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Research Article

FORMULATION AND EVALUATION OF TIMOLOL MALEATE OPHTHALMIC SOLUTION USING VARIOUS PRESERVATIVES

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Abstract

Preservatives provide important and necessary antimicrobial activity and plays very important role in maintaining the sterility and shelf-life of multi-dose formulations of topical ophthalmic medications. Any drop delivered in a multi-dose format must have some mechanism for maintaining the sterility of the contents throughout its intended length of use. In topical preparations, antimicrobial activity is most often achieved through the addition of preservatives. The most commonly used preservative in topical drops of any form is benzalkonium chloride (BAK). Whilst it is known to be an effective antimicrobial agent, demonstrating efficacy against a wide variety of common pathogens, considerable evidence, often from its use in glaucoma medications, also exists detailing the deleterious effects it has on the ocular surface, particularly when used over an extended period of time. It can be argued that the undesirable effects of BAK have contributed to a movement into preservative-free topical preparations. BAK is quaternary ammonium compound and this compound has been shown to cause tear film instability, loss of goblet cells, conjunctival squamous metaplasia and apoptosis, disruption of the corneal epithelium barrier, and damage to deeper ocular tissues.

The intend of the present study was to formulate a formulation for Timolol Maleate (0.5%) ophthalmic solution using different concentration of different preservatives to know other alternatives of Benzalkonium Chloride and which is less harmful than Benzalkonium Chloride. While selecting the type and concentration of preservatives, it was considered that added quantity of preservative must meet compendial requirement of Preservative Efficacy Testing. Various preservatives like Benzalkonium chloride, Phenyl Mercuric Nitrate, Chlorbutol and Stabilised Oxychlorocomplex were used. All the formulations were evaluated and checked for effective antimicrobial activity without interference with the mechanism of action of the active ingredient.

Keywords: Timolol Maleate, Ocular Drug Delivery, Preservatives, Ocular Surface Tolerance, Adverse Effects, Ocular Preservative Toxicity

1. Introduction

Ophthalmic preparations are dosage forms which are designed to be instilled onto the external surface of the eye (topical), administered inside (intraocular),

adjacent to the eye (per ocular) or used in conjunction with any special device. Delivery of medicine to the human eye is an integral part of medical treatment[1]. The anatomy, physiology, and biochemistry of the eye render this organ highly impervious to foreign substances hence ophthalmic drug delivery is one of the most exciting and tough endeavors facing the pharmaceutical scientist. 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 formulations may have any several purposes like healing, therapeutic, prophylactic or palliative. The versatility of dosage form enables therapeutic agent to be suitable for function of preparation. Generally used topical ophthalmic therapeutic dosage forms are solutions and suspensions.

Ophthalmic formulations are most often multi-dose products which contain suitable preservatives. Use of preservatives is done to justify their long-term use but it also represents a risk for the ocular surface. Preservatives are added to topical ophthalmic drug solutions in order to prevent contamination with pathogenic organisms. Preservatives used in ocular formulations are categorized into two broad categories: detergents and oxidizing agents. More recently, ionic-buffer system preservatives have been introduced.

Many preservatives are used in various formulations of ophthalmic preparations but benzalkonium chloride (BAK) is the most commonly used preservative[3]. BAK is a quaternary ammonium compound that exerts its microbicidal action by disrupting the microorganism's cell membranes. It was first introduced way back in 1910 as a germicide but it soon found a wide range of uses. By 1940 it was being used in toilet products such as: aftershave, mouthwash, hand washes, laundry detergents, softeners for textiles, deodorants, hair products, sanitizers, in medical products like nasal sprays, spermicides, disinfectants for surgical instruments, burn and ulcer treatment, preoperative skin disinfectants and so on.

Initially Benzalkonium chloride was used in ophthalmic solutions for preserving hard contact lenses. BAK is undoubtedly the most frequently used preservative in topical eye drops today which is used in concentration ranges from 0.004% to 0.02%. BAK is used frequently as a preservative because of its extreme efficacy in tackling microbial contamination of eye drops and its capability to disrupt cell–cell junctions in the corneal epithelium, thus allowing drug particles of topical eye drops to enter the anterior chamber. Besides, on being used as preservative for such a long time, BAK is very common to those who are formulating topical ophthalmic drug solutions. Hence is the most commonly used preservative in eye drop formulations[3].

Most of the literature and peer-reviewed articles on preservative toxicity focuses on BAK as it is the most commonly used preservative and perhaps is the most toxic of all. BAK can accumulate in ocular tissues and induce changes in the conjunctiva and cornea which may manifest as an ocular surface disease (OSD). OSD occurrence is reportedly as high as thrice that of the general population in patients on chronic anti-glaucoma medications[4].

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 0.02% to 0.004% 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 different preservatives to know other alternatives of Benzalkonium Chloride and which preservative is less harmful than Benzalkonium Chloride and to minimize the concentration of Benzalkonium Chloride. While selecting the concentration of Benzalkonium chloride and other preservatives, it must be keep in mind that added quantity of preservative must meet compendial requirement of Preservative Efficacy Testing[5]. Various preservatives used were Benzalkonium chloride, Phenyl Mercuric Nitrate, Chlorbutol and Stabilised Oxychlorocomplex.

2. Material and Instruments

Various materials and Instruments used in experimental work are listed below in Table 1 and Table 2.

Various Instruments used in experimental work are listed in Table 2.

Table 1: List of Materials 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 Packaging's, Bangalore

Table 2: List of Instruments Used

S.No.	Name of Material	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.	Hot air oven	Alpha scientific, Bangalore
13.	Incubator	Servewell instruments, Pvt. Ltd, Bangalore

3. Material and Instruments

3.1 Prototype Formulation Development

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

The proposed formula was optimized by varying the preservative of three different concentrations. The quantities of Timolol maleate and other excipients were kept constant. The aim of the present study was to optimize the preservative & their concentration in formulation for Timolol maleate (0.5%) ophthalmic solution.

Table 3: Prototype Formulation

S. No.	Name of Excipient	Category	Uses
1.	Benzalkoniumchloride	Preservative	Benzalkonium chloride prevents bacterialand 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.	MonobasicSodium Phosphate	Buffering agent, Sequestering agent, Emulsifying agent	Buffering agent and electrolyte replenisher, when combined withother phosphates
4.	Sodium hydroxide	Alkali	For pH adjustment

Table 4: Composition of Timolol Maleate Ophthalmic Solution using Benzalkoniumchloride

Name of Ingredient	Formulation Batches		
	TIM/A01	TIM/A02	TIM/A03
Timolol Maleate	0.5%w/v	0.5%w/v	0.5%w/v
Benzalkoniumchloride	0.016%v/v	0.02%v/v	0.024% v/v
EDTA Sodium	1.0 mg/ml	1.0 mg/ml	1.0 mg/ml
Disodium Hydrogen Phosphate	3.0 mg/ml	3.0 mg/ml	3.0 mg/ml
Sodium AcidPhosphate	5.75mg/ml	5.75mg/ml	5.75mg/ml
Sodium Chloride	5.0 mg/ml	5.0 mg/ml	5.0 mg/ml
Sodium hydroxide	QS to adjust pH	QS to adjust pH	QS to adjust pH
Water for Injection	QS	QS	QS

Table 5: Composition of Timolol Maleate Ophthalmic Solution using Phenyl Mercuric Nitrate

Name of Ingredients	Formulation Batches		
	TIM/A01	TIM/A02	TIM/A03
Timolol Maleate	0.5%w/v	0.5%w/v	0.5%w/v
Phenyl MercuricNitrate	0.001%v/v	0.0012%v/v	0.0014% v/v
EDTA Sodium	1.0 mg/ml	1.0 mg/ml	1.0 mg/ml
Disodium Hydrogen Phosphate	3.0 mg/ml	3.0 mg/ml	3.0 mg/ml
Sodium Acid Phosphate	5.75 mg/ml	5.75 mg/ml	5.75 mg/ml
Sodium Chloride	5.0 mg/ml	5.0 mg/ml	5.0 mg/ml
Sodium hydroxide	QS to adjust pH	QS to adjust pH	QS to adjust pH
Water for Injection	QS	QS	QS

Table 6: Composition of Timolol Maleate Ophthalmic Solution using Chlorbutol

Name of ingredients	Formulation Batches		
	TIM/A01	TIM/A02	TIM/A03
Timolol Maleate	0.5%w/v	0.5%w/v	0.5%w/v
Chlorbutol	0.045%v/v	0.500%v/v	0.550% v/v
EDTA Sodium	1.0 mg/ml	1.0 mg/ml	1.0 mg/ml
Disodium Hydrogen Phosphate	3.0 mg/ml	3.0 mg/ml	3.0 mg/ml
Sodium Acid phosphate	5.75mg/ml	5.75 mg/ml	5.75 mg/ml
Sodium Chloride	5.0 mg/ml	5.0 mg/ml	5.0 mg/ml
Sodium hydroxide	QS to adjust pH	QS to adjust pH	QS to adjust pH
Water for Injection	QS	QS	QS

Table 7: Composition of Timolol Maleate Ophthalmic Solution using Stabilised Oxychloro Complex

Name of ingredients	Formulation Batches		
	TIM/A01	TIM/A02	TIM/A03
Timolol Maleate	0.5%w/v	0.5%w/v	0.5%w/v
Stabilised Oxychloro Complex	0.045%v/v	0.500%v/v	0.550% v/v
EDTA Sodium	1.0 mg/ml	1.0 mg/ml	1.0 mg/ml
Disodium Hydrogen Phosphate	3.0 mg/ml	3.0 mg/ml	3.0 mg/ml
Sodium AcidPhosphate	5.75 mg/ml	5.75 mg/ml	5.75 mg/ml
Sodium Chloride	5.0 mg/ml	5.0 mg/ml	5.0 mg/ml
Sodium hydroxide	QS to adjust pH	QS to adjust pH	QS to adjust pH
Water for Injection	QS	QS	QS

Batches were planned by taking four different preservatives of three different concentrations:

A. Benzalkonium chloride (0.016%, 0.020%, 0.024 % v/v).

B. Phenyl mercuric Nitrate (0.001%w/v, 0.0012% w/v, 0.0014% w/v).

C. Chlorbutol (0.45% w/v, 0.50% w/v, 0.55% w/v).

D. Stabilised Oxychlorocomplex (0.0045% w/v, 0.005% w/v, 0.0055% w/v)

3.2 Preservative Efficacy Test (PET) for Timolol Maleate 0.5% Ophthalmic Solution [5,6]

Sample from all twelve batches were subjected to preservative efficacy test of Benzalkonium chloride, Phenyl Mercuric Nitrate, Chlorbutol and Stablised Oxychloro Complex in Timolol maleate 0.5% ophthalmic solution. The most stringent criteria of Indian pharmacopoeia were followed for experiment.

3.3 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.

3.4 Test Procedure for Anti-Infective Effectiveness

The product had been transferred to five sterile, capped bacteriological containers. Each container was inoculated with one of the prepared and

standardized inoculums and mixed. The volume of suspension inoculums was between 0.5% and 1.0% of the volume of product. 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. Sample was incubated at 22.5 ± 25^{0}C. The initial concentration of viable microorganisms was determined by plate count method. Each container was sampled at intervals of 0 hrs, 6 hrs, 24 hrs, 7 days, 14 days, 21 days, and 28 days for different microorganisms. Sample was extracted into neutralizing fluid to inactivate residual preservative 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[7,8,9,10].

Microbial Count (cfu/ml) of Product was calculated by using following formula:

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

$$\textit{Log reduction} = \textit{Log of initial count} - \textit{Log of final count}$$

4. Results and Discussions

4.1 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.

Tests	Result
Description	A white powder
Solubility	Soluble in ethanol(95%) & in Water, sparinglysoluble in chloroform, insoluble in ether
Identification by Chemically	Color changes to Violet Blue
Appearance of Solution	2.0% w/v solution in CO2 free water is clear
pH	4.1
Specific Optical Rotation	- 5.8
Sulphated Ash	0.06%
Loss on Drying	0.11%
Assay by Titration	99.9%

4.2 Finished Product Analysis

Tests	Result
Description	A white powder
Identification by Chemically	Soluble in ethanol (95%) & in water, sparingly soluble in chloroform, in-soluble in ether
Clarity	2.0% w/v solution in CO_2 free water is clear
pH	Color changes to Violet Blue
Sterility	Sterile

4.3 Assay by UV

Batch No.	Potency
TIM/A01	101.82%
TIM/A02	101.58%
TIM/A03	101.00%
TIM/B01	92.79%
TIM/B02	92.72%
TIM/B03	92.74%
TIM/C01	96.31%
TIM/C02	96.83%
TIM/C03	96.96%
TIM/D01	98.82%
TIM/D02	98.86%
TIM/D03	99.01%

Figure 1: Finished product of various batches

4.4 Results of Preservative Efficacy Test:

Table 8: Log Reduction in Bacterial Growth

BAK CONCENTRATION	OBSERVATION (LOG REDUCTION)					
	6 HRS	24 HRS	7^{th} DAY	14^{th} DAY	21^{th} DAY	28^{th} DAY
0.016%	1	2	2	2	2	2
0.020 %	2	3	5	5	5	5
0.024 %	2	3	5	5	5	5
Acceptance criteria	*Min 2 Log Reduction*	*Min 3 Log Reduction*	-	-	-	*No Recovery*

PHENYL MERCURIC CONCENTRATION	OBSERVATION (LOG REDUCTION)					
	6 HRS	24 HRS	$_{7}$th DAY	$_{14}$th DAY	$_{21}$th DAY	28th DAY
0.001%	2	3	5	5	4	4
0.0012 %	2	5	5	5	5	5
0.0014 %	2	5	5	5	5	5

CHLORBUTOL CONCENTRATION	OBSERVATION (LOG REDUCTION)					
	6 HRS	24 HRS	$_{7}$th DAY	$_{14}$th DAY	$_{21}$th DAY	28th DAY
0.045%	1	3	5	5	5	5
0.500%	2	2	5	5	5	5
0.550 %	2	5	5	5	5	5

STABILISED OXYCHLOROCOMPLEX CONCENTRATION	OBSERVATION (LOG REDUCTION)					
	6 HRS	24 HRS	$_{7}$th DAY	$_{14}$th DAY	$_{21}$th DAY	28th DAY
0.045%	1	2	5	5	5	5
0.500 %	2	5	4	4	4	4
0.550 %	2	3	4	5	5	5

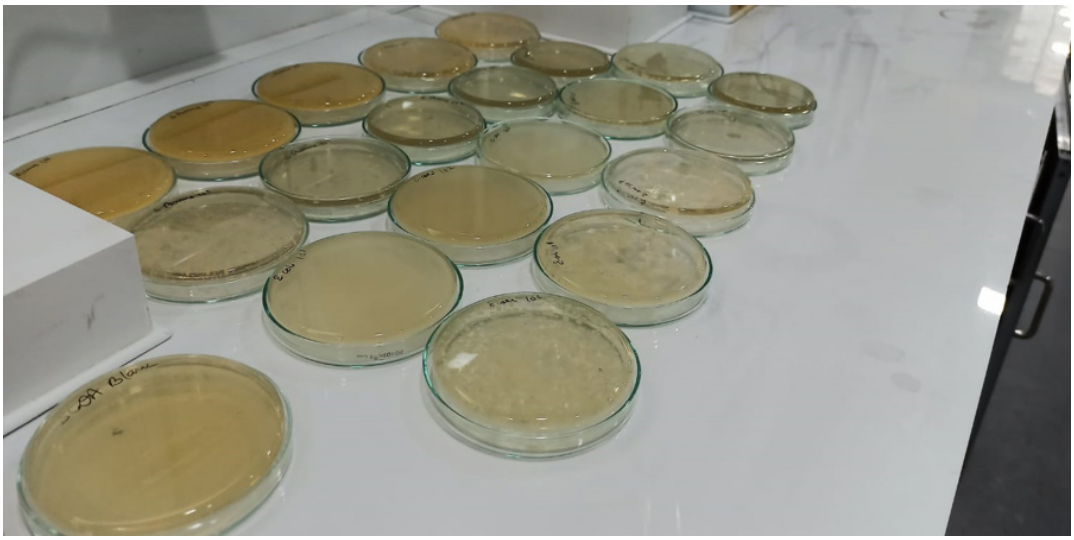

Figure 2: The antimicrobial efficacy test

Results for Fungal log reduction with different concentration are summarized in following table.

Table 9: Log Reduction in Fungal Growth

BAK CONCENTRATION	OBSERVATION (LOG REDUCTION)					
	6 HRS	24 HRS	$_{7}$th DAY	$_{14}$th DAY	$_{21}$th DAY	28th DAY
0.016%	0	0	1	1	1	1
0.020 %	0	1	2	3	3	3
0.024 %	0	1	2	3	3	3
Acceptance criteria	-	-	Min 2 Log Reduction	-	-	No Recovery

PHENYLMERCURIC CONCENTRATION	OBSERVATION (LOG REDUCTION)					
	6 HRS	24 HRS	$_{7}$th DAY	$_{14}$th DAY	$_{21}$th DAY	28th DAY
0.001%	0	0	0	1	1	1
0.0012 %	0	0	1	2	2	2
0.0014 %	0	1	2	3	4	4

CHLORBUTOL CONCENTRATION	OBSERVATION (LOG REDUCTION)					
	6 HRS	24 HRS	$_{7}$th DAY	$_{14}$th DAY	$_{21}$th DAY	28th DAY
0.045%	0	1	2	3	3	3
0.500%	0	1	2	2	2	2
0.550 %	0	2	3	4	4	4

STABILISED OXYCHLORO COMPLEX CONCENTRATION	OBSERVATION (LOG REDUCTION)					
	6 HRS	24 HRS	$_{7}$th DAY	$_{14}$th DAY	$_{21}$th DAY	28th DAY
0.045%	0	1	1	1	1	1
0.500 %	0	2	3	3	4	4
0.550 %	0	2	3	3	3	3

4.5 The results obtained from the experiment, in which

Benzalkonium chloride (0.016%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 (0.016%v/v) showed one log reduction at 7th day whereas criteria states that reduction must be of 2 log at 7th day.

Benzalkonium chloride (0.020% v/v) showed 2 log reductions at 6 hours and 3 log reductions at 24 hours and no recovery at 28th day for bacteria. For fungi it showed log reduction as stated in criteria, 2 log reductions at 7th day and no recovery at 28th day.

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 28th day means there was no recovery of bacteria. For fungi Benzalkonium chloride (0.024% w/v strength) reduced fungus count by 2 log at 7th day. It also showed 4 log reductions at 28th day. Hence Benzalkonium chloride (0.024% v/v) passes both for bacteria but and fungi.

Phenyl Mercuric Nitrate (0.001% v/v) passed criteria for bacteria for 2 log and 3 log reductions at 6 hours and 24 hours. For fungi, it failed to reduce fungus count by 2 log at 7th day. It showed only 1 log reduction. Hence Phenyl Mercuric Nitrate (0.001% v/v) failed for both bacteria & Fungi.

Phenyl Mercuric Nitrate (0.0012% v/v) passed criteria for bacteria for 2 log and 3 log reductions at 6 hours and 24 hours. For fungi, it failed to reduce fungus count by 2 log at 7th day. It showed only 1 log reduction. Hence Phenyl Mercuric Nitrate (0.001% v/v) failed for both bacteria & Fungi.

Phenyl Mercuric Nitrate (0.0014% v/v) passed criteria for bacteria for 2 log reductions at 6 hours but failed for 3 log reduction at 24 hours . For fungi, it failed to reduce fungus count by 2 log at 7th day. It showed only 1 log reduction. Hence Phenyl Mercuric Nitrate (0.0014% v/v) failed for both bacteria & Fungi.

Chlorbutol (0.045% v/v) passed criteria for bacteria for 3 log reductions at 24 hours but failed for 2 log reduction at 6 hours . For fungi, it passed criteria for fungi 2 log reduction at 7th day. Hence Chlorbutol (0.045% v/v) failed for bacteria & passed for Fungi.

Chlorbutol (0.500% v/v) passed criteria for bacteria for 2 log reductions at 6 hours but failed for 3 log reduction at 24 hours . For fungi, it passed criteria for fungi 2 log reduction at 7th day. Hence Chlorbutol (0.045% v/v) failed for bacteria & passed for Fungi.

Chlorbutol (0.550% v/v) passed criteria for bacteria for 2 log reductions at 6 hours but failed for 3 log reduction at 24 hours . For fungi, it failed for 2 log reduction at 7th day. Hence Chlorbutol (0.045% v/v) failed for both bacteria & Fungi.

Stabilised Oxychloro Complex (0.045% v/v) passed criteria for bacteria for 2 log reductions and 3 log reduction at 6 hours & 24hrs respectively but there is recovery at 28th day . For fungi, it failed for 2 log reduction at 7th day. Hence Stabilised Oxychloro Complex (0.045% v/v) failed for both bacteria & Fungi.

Stabilised Oxychloro Complex (0.500% v/v) passed criteria for bacteria for 2 log reductions at 6 hours but failed for 3 log reduction at 24hrs. For fungi, it failed for 2 log reduction at 7th day. Hence Stabilised Oxychloro Complex (0.500% v/v) failed for both bacteria & Fungi.

Stabilised Oxychloro Complex (0.550% v/v) passed criteria for bacteria for 2 log reductions and 3 log reduction at 6 hours & 24hrs respectively but there is recovery at 28th day. Hence Stabilised Oxychloro Complex (0.550% v/v) failed for both bacteria & Fungi.

5. Summary and Conclusion

5.1 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 Indian Pharmacopeia 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[11].

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[12].

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

The proposed formula was optimized by varying the concentration of preservative. 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 of preservative. For all twelve batches *Preservative*

Efficacy Testing was carried out according to Indian Pharmacopoeia[5].

5.2 Conclusion

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 28thday for bacteria. For fungi it showed log reduction as stated in criteria, 2 log reductions at 7th day and no recovery at 28th day. Both these concentrations passed the criteria according to Indian 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.

Conflict of Interest: None

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