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Effect and safety of Benzalkonium chloride in an ophthalmic solution containing Ofloxacin

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ABSTRACT

The study was planned to determine the safest and the most effective concentration of Benzalkonium Chloride (BAK) in an ophthalmic solution containing antibiotic ofloxacin. To determine the safe and effective concentrations of BAK, three different formulations of ophthalmic solution were prepared by using the various concentrations of BAK. The preservative efficacy test (PET) was conducted for all the formulations. PET was performed according to British Pharmacopoeia. British Pharmacopoeia states that there must be 2 log reductions at 6 Hrs and 3 log reductions at 24 Hrs with no recovery at 28th days for bacteria. For fungi there must be 2 log reductions at 7th day with no recovery at 28th days. The results showed that 0.010 v/v concentration failed for required log reductions at 6 Hrs, 24 Hrs for bacteria and at 7th day for fungi. 0.016% v/v concentration passed criteria for log reduction for bacteria but failed for log reduction of fungi. 0.020% v/v concentration passed criteria for both bacteria and fungi.

Keywords: Ofloxacin, Benzalkonium chloride, Preservative efficacy test, Log reductions.

INTRODUCTION

Topically applied ophthalmic products, regardless of their use, usually contain water for injection as one of primary component. The 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 need preservative system. Preservative system can be either a single agent or combination of agents. [1]

Anti-infective ophthalmic solution: To Preserve or Not to Preserve? : In ophthalmology, preservative can act as a both friend and foe. We rely on them to protect against microbial contamination while remaining vigilant for rare instances of cytotoxicity. Equally important is the need to recognize little known benefits, besides protecting against contamination.

Addition of preservative agent increases protection from contamination and more antimicrobial effect, resulting in cidal activity and more rapid rate of killing organisms. If we do not use preservative in formulation larger amount of drug will be expended over product protection and less will remain for therapeutic applications. For that reason a preservative must be added to the formulation to protect the drug. [2]

Ofloxacin ophthalmic solution (0.3%) which contains preservative is widely used though many preservative free fluoroquinolone preparations have been developed.

Commonly used preservatives used in Ophthalmics: Specific chemicals which are toxic to micro-organisms may be added in products, which don't have any protecting agents in it and for which contamination with undesirable micro-organisms is possible if used multiple number of times. e.g. multidose vials, eye drops etc.

The most common used ocular preservative Benzalkonium Chloride (BAK) enhances a medication absorption rate, in some cases increases the speed of microbial killing, and prevents contamination of the product. The various preservatives used in ophthalmic preparation used are summarized in table 1.

Table 1

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

It is found that near about 72% of marketed ophthalmic solutions are preserved with BAK whereas only 20% formulations contain other preservatives and just 8% of ophthalmic solutions are preservative free.

About BAK: BAK has endured a barrage of criticism but continuous to maintain its position as gold standard for preservatives in topical ophthalmic medication.

BAK is quaternary ammonium compound that acts as a detergent preservative. It works by adhering to the cell membrane of micro-organism and increases their permeability which leads to cell lysis.

Preservatives have come under scrutiny of research studies over the years for their potential ability to disrupt the protective tear film and ocular surface cell.[3] It is reported that concentration from 0.1% to 0.0001% induce dose dependent growth arrest and cell death, either delayed or immediately after administration.

The studies showing the effect of BAK exposure have yielded statistically significant differences on ocular surface with the use of other preservatives. Several studies used animal or isolated cells or in vitro models that allow the preservative to remain on ocular cell surface for extended period from several minutes to an hour. This is a poor simulation of living human eye with enact tearing and nasolacrimal drainage system. Separate studies have shown that when used on normal regimen, BAK is not toxic to corneal epithelial cell at non-exaggerated concentration. [4]

To avoid the risk of toxicity and to achieve the better biocompatibility with a product protection at safe and effective concentration of BAK in Ofloxacin 0.3% ophthalmic solution PET is recommended.

MATERIALS AND METHODS

1. Drug: Ofloxacin was used of *Neuland Laboratories Ltd.*

2. 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

3. 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.

4. Instrument: Colony counter of *Alpha Scientific.*

The proposed formula was optimized by varying the concentration of BAK. The aim of the present study was to optimize the concentration of BAK in formulation for Ofloxacin (0.3%) ophthalmic solution. Batches were planned by taking different concentrations viz. 0.01%, 0.016%, and 0.02% of BAK, Ofloxacin 0.3%, Disodium edetate, Sodium chloride, Sodium hydroxide and Hydrochloric acid to adjust pH between 6.3 and 6.5 and volume was made up by water for injection.

Test Procedure for Anti-Infective Effectiveness Test: [5] The product had been transferred to five sterile, capped bacteriological containers. Each of the containers was inoculated with one of the prepared and standardized inoculums, and was mixed. The volume of suspension inoculum 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 ± 25^0C. The

initial concentration of viable microorganisms was determined by plate count method. Each container was sampled at intervals of 0 hrs, 6hrs, 24 hrs, 7 days, 14 days, and 28 days for different microorganisms.

Using the calculated concentration of cfu/mL present at the initial of the test, calculate the change in $\log_{10}$ values of the concentration of cfu/mL for each micro-organism at the applicable test intervals, and express the changes in terms of log reduction.

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

$$\text{cfu/mL of Product} = \frac{\text{mL of the inoculum of the product suspension taken} \times \text{cfu/mL of inoculum}}{\text{Volume of reconstituted product}}$$

Table 2: Criteria for Tested Micro-Organisms as per BP

Sr. 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 log at 7 days with no increase at 28 days.

RESULTS AND DISCUSSION

Calculations for Log reduction

Table 3: Results for Bacteria

BAK Concentration (% v/v)	Observations (Log Reduction)					
	6 Hrs	24 Hrs	7 Day	14 Day	21 Day	28 Day
0.010	1	2	5	5	5	5
0.016	2	3	5	5	5	5
0.020	2	5	5	5	5	5

Table 4: Results for Fungi

BAK Concentration (% v/v)	Observations (Log Reduction)					
	6 Hrs	24 Hrs	7 Day	14 Day	21 Day	28 Day
0.010	0	0	1	1	1	1
0.016	0	1	1	2	2	2
0.020	0	1	2	3	4	4

Preservative efficacy test was performed by plate count method, for Ofloxacin 0.3% ophthalmic solution with 0.01 %v/v, 0.016% v/v and 0.02% with reference to British Pharmacopoeia, The results obtained from experiment in which 0.01 %v/v concentration failed for 2 log reduction at 6 hours and 3 logs at reduction at 24 hours for bacteria. For fungi this concentration showed only 1

log reduction where as criteria states that reduction must be of 2 log at 7th day.

0.016% v/v concentration of BAK, passes criteria for 2 log and 3 log reduction in bacterial count at 6 hours and 24 hours respectively. There were 5 log reductions at 28 days. For fungi, 0.016% v/v concentration of BAK failed to reduce fungal count by 2 logs. It showed only 1 log reduction at 7th day. Hence 0.016% v/v concentration of BAK passes only for bacteria but not for fungi.

0.02% v/v concentration of BAK showed 2 log and 5 log reduction for bacteria at 6 hours and 24 hours respectively and no recovery at 28th days. For fungi this concentration showed log reductions as stated in criteria; 2 log reductions at 7th day and no recovery at 28th day. This concentration showed 4 log reductions at 28th day.

CONCLUSION

In ofloxacin 0.3% ophthalmic solution 0.02% v/v concentration of BAK showed excellent results for inhibition of growth for both bacteria and fungi. Above 0.02% v/v BAK may cause problem of eye irritation of the corneal membrane and hence it is concluded that in ofloxacin 0.3% ophthalmic solution, 0.02% v/v concentration of BAK is the safest and most effective concentration.

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