Evaluation of Antimicrobial Preservative Efficancy Test of Benzalkonium Chloride as IPC

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ABSTRACT:
The effective preservation of pharmaceutical products against microbial growth is crucial for ensuring consumer safety and maintaining antimicrobial activity during manufacturing and storage. In this study, we evaluated the antimicrobial preservation efficacy of Benzalkonium solution in ciprofloxacin eye drops, following the guidelines established by the Indian Pharmacopoeia (IPC).
To assess the preservative effectiveness, we inoculated the product with a test microorganism and stored it at room temperature (27°C) for 28 days. We then used the duplication plate count method to determine the number of viable microbes present at each specified time interval.
Our results shed light on the importance of effective antimicrobial preservation methods in pharmaceutical products, particularly those containing Benzalkonium solution in ciprofloxacin eye drops. Our findings can aid the pharmaceutical industry in developing and implementing safe and effective preservation strategies to maintain product quality and safety.

ABBREVIATIONS: SCDA: Soyabean Casein Digest Agar, SCDM: Soyabean Casein Digest Agar, SDA: Sabouraud Dextrose Agar, TYMC: Total yeast and Mold count, TAMC: Total aerobic microbial count, ATCC: American Type Culture Collection

KEYWORDS: Colony-forming units (CFU), dose parental, ophthalmic, antimicrobial, Benzalkonium chloride, and IPC (Indian pharmacopoeia commission).

INTRODUCTION

Ciprofloxacin drops are sterile pharmaceutical products that contain components that provide an ideal source of growth for microorganisms. As such, suitable preservatives are added during manufacturing to prevent microbial contamination, which can lead to infections in consumers.Antimicrobial preservatives not only minimize microorganism counts but also control their multiplication.

The purpose of this study was to evaluate the efficacy of Benzalkonium Chloride as an antimicrobial preservative, as per the guidelines established by the Indian Pharmacopoeia (IPC). To protect consumers, the preservative was added to the final package at a low concentration.

To ensure the preservation of antimicrobial activity during storage, the Antimicrobial Preservative Efficacy test was performed on multiple dose parenteral, nasal, oral analgesic products prepared with an aqueous base. This test involved inoculating the preparation with a specific microorganism and maintaining it at a particular temperature for a period of 28 days. At specified intervals (0, 7, 14, and 28 days), the sample was removed and the number of organisms was counted using the pour plate method.

The product was inoculated with three bacteria and two fungi as challenge organisms. The results of the study contribute to our understanding of the importance of effective antimicrobial preservation methods in pharmaceutical products and demonstrate the efficacy of Benzalkonium Chloride as an antimicrobial preservative.

The study provides important insights that can help the pharmaceutical industry develop and implement safe and effective preservation strategies, ensuring the safety and efficacy of products for consumers.

MATERIALS AND METHODS

Sample: This sample consist of Benzalkonium chloride solution as antimicrobial preservative in ciprofloxacin eye drop.

Test microorganism:

- *Pseudomonas aeruginosa ATCC 9027*
- *Staphylococcus aureus ATCC 6528*
- *Candida albicans ATCC 10231*
- *Aspergillus brasillensis ATCC 16404*
- *Escherichia coli ATCC 8739*

Test method

This test is done by Pour Plate Method ,The test organism is cultivated in growth medium, use Soyabean Casein Digest Agar Medium for Bacterial culture while Sabouraud Dextrose Agar Medium used for the recovery of Fungus and Yeast, the media were sterilized in the autoclave at 121°c and 15psi and 15 mint and all standard microbial stains collected from American type culture collection(ATCC), were preserved as lyophilized and glyceried stocks.

Preparation of inoculums

Each test organism have stock culture for subculture on the surface of suitable specific media the bacterial culture are incubate at 30°-35°C for 18-24hrs and incubate the culture of fungus at 20°-25°c for 48hrs.

Methodology

The efficacy of antimicrobial preservative was tested in accordance with the protocol described in the Indian Pharmacopoeia. A culture suspension containing no less than 10^6 CFU was added to a sterilized test tube containing 20 mL of ciprofloxacin eye drop sample, with the suspension not exceeding 1% of the volume of the product. This constituted the stock solution, which was diluted to 10^-1 to obtain a countable colony.8

Next, 1 mL of the suspension was transferred into a petri dish based on their dilution in the respective media plate using the pour plate method. The plates were incubated at temperatures ranging from 30°C-35°C for 3-5 days for SCD A and 20°C-25°C for 5-7 days for SDA. Visible cell counts were periodically determined at intervals of 0, 7, 14, and 28 days, as shown in the following table:

Table No.1 Observation of test peformed on "0" day

MICROORGANISM	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Escherichia coli	nil	nil	nil	nil	1	98	5	Nil
Staphylococcus aureus	nil	nil	nil	nil	nil	nil	nil	Nil
Psedumonas aeruginosa	nil	nil	nil	nil	nil	nil	nil	Nil
Candida albicans	TNTC	TNTC	TNTC	TNTC	36	1	nil	-
Aspergillus brasiliensis	TNTC	TNTC	8	1	1	1	-	-

Table No.2 Obsevation of test performed on "7th" day

MICROORGANISM	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Escherichia coli	nil	nil	nil	nil	nil	nil	nil	Nil
Staphylococcus aureus	nil	nil	nil	nil	nil	nil	nil	Nil
Psedumonas aeruginosa	nil	nil	nil	nil	nil	nil	nil	Nil
Candida albicans	nil	nil	nil	nil	nil	nil	nil	0
Aspergillus brasiliensis	nil	nil	nil	nil	nil	nil	-	-

Table No.3 Observation of test performed on "14" day

MICROORGANISM	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-8}	10^{-9}
Escherichia coli	nil	nil	nil	nil	nil	nil	nil	Nil
Staphylococcus aureus	nil	nil	nil	nil	nil	nil	nil	Nil

Psedumonas aeruginosa	nil	nil	nil	nil	nil	nil	nil	Nil
Candida albicans	nil	nil	nil	nil	nil	nil	nil	-
Aspergillus brasiliensis	nil	nil	nil	nil	nil	nil	-	-

Table No.4 Observation of test performed on "28" day

MICROORGANISM	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Escherichia coli	nil	nil	nil	nil	nil	nil	nil	nil
Staphylococcus aureus	nil	nil	nil	nil	nil	nil	nil	nil
Psedumonas aeruginosa	nil	nil	nil	nil	nil	nil	nil	nil
Candida albicans	nil	nil	nil	nil	nil	nil	nil	-
Aspergillus brasiliensis	nil	nil	nil	nil	nil	nil	-	-

RESULT

The antimicrobial preservative efficacy of a Benzalkonium Chloride solution in Ciprofloxacin eye drops was evaluated. The results showed that the suspension did not exceed the acceptable microbial limit for bacteria and fungi throughout the 28-day incubation period. This indicates that the preservative used in the tested eye drops effectively demonstrated antimicrobial activity against microorganisms. In conclusion, this study suggests that the preservative utilized in the Ciprofloxacin eye drops could be a suitable option for maintaining the product's antimicrobial activity and ensuring the safety of consumers.

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CONCLUSION

The effect of preservative examined in the product, the concentration of the viable bacteria is not more than 0.1% of the initial concentration by the 14^{th} days and the concentration of the yeast and moulds remain below the concentration of each test microorganism remain below the level during the remainder of the 28 days test period. In order to protect the consumer, introduce in the final packaging low concentration of the preservative that may be toxic to human beings.

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