

# Formulation and Development of Ketorolac Tromethamine Ophthalmic Solution

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## Abstract

Ophthalmic preparations are specialized dosage forms designed to be instilled on to the external surface of 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. Ketorolac tromethamine is a Nonsteroidal anti-inflammatory drug. It is used as Antipyretic, anti-inflammatory and analgesic. It is indicated Ketorolac tromethamine ophthalmic solution is indicated for the temporary relief of ocular itching due to seasonal allergic conjunctivitis. Ketorolac tromethamine ophthalmic solution is also indicated for the treatment of postoperative inflammation in patients who have undergone cataract extraction. Therefore, the aim of the present study was to formulate a formulation for ketorolac tromethamine (0.5%) ophthalmic solution using different concentration of Benzalkonium chloride as preservative. While reducing the concentration of Benzalkonium chloride it must be kept in mind that added quantity of preservative must meet compendial requirement of Preservative Efficacy Testing as per United State Pharmacopoeia (USP). The present research work is also planned to provide the data about the selection of suitable primary packaging material for Ketorolac tromethamine (0.5%) 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.

**Keywords:** Ophthalmic solution; Ketorolac; Preservative; Incompatibility; Stability; Concentration

## 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 on to the external surface of 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 residence time of an ocular preparation may range from few seconds (ophthalmic solutions) to hours (gel, ointments), two months or years (intra ocular or periocular dosage forms). 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. Widely used topical ophthalmic therapeutic dosage forms are solutions and suspensions. Ophthalmic solutions are most often multidose product containing suitable preservative(s) to meet compendial Preservative Efficacy Test (United State Pharmacopoeia, British Pharmacopoeia, Japanese Pharmacopoeia and Indian Pharmacopoeia) requirements [3].

Ketorolac tromethamine is a nonsteroidal anti-inflammatory drug. It is used as Antipyretic, anti-inflammatory and analgesic. It is indicated Ketorolac tromethamine ophthalmic solution is indicated for the

temporary relief of ocular itching due to seasonal allergic conjunctivitis. Ketorolac tromethamine ophthalmic solution is also indicated for the treatment of postoperative inflammation in patients who have undergone cataract extraction [4,5]. 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 [6].

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 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 [7].

Benzalkonium chloride (BAK) belongs to Quaternary ammonium compound class used as preservative in ophthalmic solution. Kill

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microorganisms by disrupting cell membranes and causing cell lyses. It should be used in very low concentration otherwise harmful to cell. 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 [7].

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 [7]. Acetic acid derivatives ketorolac tromethamine has found its applicability in both gram positive and gram negative bacterial ocular infection and used 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 ketorolac tromethamine (0.5%) ophthalmic solution using different concentration of Benzalkonium chloride as preservative. While reducing the concentration of Benzalkonium chloride it must be kept in mind that added quantity of preservative must meet compendial requirement of Preservative Efficacy Testing as per United States Pharmacopoeia (USP).

The present research work is also planned to provide the data about the selection of suitable primary packaging material for Ketorolac tromethamine (0.5%) 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.

## Materials and Methods

### Materials

Ketorolac tromethamine was obtained from MSN Laboratories Ltd, Medak, India. Benzalkonium chloride, Sodium chloride, Disodium EDTA, Octoxynol-40, Sodium hydroxide and Hydrochloric acid were obtained from Merck Ltd, Mumbai, India. Growth Media and Neutralizer media were obtained from High Media Ltd, Mumbai, India.

### Methods

**Formulation studies:** Development of Ketorolac tromethamine ophthalmic solution was done in two phases. Firstly Prototype formulation was developed and then designed the final formula for manufacturing of Ketorolac tromethamine ophthalmic solution.

**Prototype formulation development:** The prototype formulation for Ketorolac tromethamine ophthalmic solution was representing in table 1.

**Formulation design:** The formula for Ketorolac tromethamine ophthalmic solution was designed. The optimization of proposed

Sr. No	Name of excipient	Category
1	Benzalkonium chloride	Preservative
2	Sodium chloride	For isotonicity
3	Disodium EDTA	Chelating agent
4	Octoxynol 40	Preservative
5	Hydrochloric acid	Acid
6	Sodium hydroxide	Alkali

**Table 1:** Prototype formulation.

Name of ingredients	Formulation batches					
	OPT/KTM/T-01	OPT/KTM/T-02	OPT/KTM/T-03	OPT/KTM/T-04	OPT/KTM/T-05	OPT/KTM/T-06
Ketorolac tromethamine	5 mg/mL	5 mg/mL	5 mg/mL	5 mg/mL	5 mg/mL	5 mg/mL
Benzalkonium chloride	0.00 mg/MI	0.05 mg/MI	0.06 mg/MI	0.08 mg/MI	0.1 mg/MI	0.12 mg/MI
Disodium EDTA	1.0 mg/MI	1.0 mg/MI	1.0 mg/MI	1.0 mg/MI	1.0 mg/MI	1.0 mg/MI
Sodium chloride	7.9 mg/MI	7.9 mg/MI	7.9 mg/MI	7.9 mg/MI	7.9 mg/MI	7.9 mg/MI
Octoxynol 40	0.1 mg/MI	0.1 mg/MI	0.1 mg/MI	0.1 mg/MI	0.1 mg/MI	0.1 mg/MI
Hydrochloric acid	adjust pH	adjust pH	adjust pH	adjust pH	adjust pH	adjust pH
Sodium hydroxide	adjust pH	adjust pH	adjust pH	adjust pH	adjust pH	adjust pH
Water for injection	QS	QS	QS	QS	QS	QS

**Table 2:** Composition of different concentration BKC in Ketorolac tromethamine Ophthalmic Solution.

Name of ingredients	Formulation batches		
	OPT/KTM/T-01	OPT/KTM/T-02	OPT/KTM/T-03
Ketorolac tromethamine	5 mg/ml	5 mg/mL	5 mg/MI
Benzalkonium chloride	0.06 mg/MI	0.08 mg/MI	0.1 mg/MI
Disodium EDTA	1.0 mg/MI	1.0 mg/MI	1.0 mg/MI
Sodium chloride	7.9 mg/MI	7.9 mg/MI	7.9 mg/MI
Octoxynol 40	0.1 mg/MI	0.1 mg/MI	0.1 mg/MI
Hydrochloric acid	adjust pH	adjust pH	adjust pH
Sodium hydroxide	pH 6.5	pH 7.4	pH 8.4
Water for injection	QS	QS	QS

**Table 3:** Composition of Ketorolac tromethamine Ophthalmic Solution change in pH stability.

formula was done by varying the concentration of Benzalkonium chloride. The quantities of Ketorolac tromethamine and other excipients were kept constant. As the aim of the present study was to optimize the concentration of Benzalkonium chloride in formulation for Ketorolac tromethamine (0.5%) ophthalmic solution (Table 2). Batches were planned by taking different concentrations viz. 0.05% v/v, 0.06% v/v, 0.08% v/v, 0.1% v/v, and 0.12% v/v of Benzalkonium chloride, Ketorolac tromethamine 0.5%, Disodium EDTA, Sodium chloride, Sodium hydroxide (Table 2) and Hydrochloric acid to adjust pH between 7.2 to 7.6 (Table 3) and volume was made up by water for injection.

**Optimized formula and manufacturing:** The Optimized formula for Ketorolac tromethamine ophthalmic solution was given in table 4.

**Manufacturing process of ketorolac tromethamine ophthalmic solution:**

1. Collect water for injection in a cleaned glass container and parching it with nitrogen gas for 30 min.

Name of ingredients	OPT/KTM/ T-0014
Ketorolac tromethamine	5 mg/mL
Benzalkonium chloride	0.08 mg/ML
Disodium EDTA	1.0 mg/ML
Sodium chloride	7.9 mg/ML
Octoxynol 40	0.1 mg/ML
Hydrochloric acid	adjust pH
Sodium hydroxide	pH 7.4
Water for injection	QS

**Table 4:** Optimized formula of Ketorolac tromethamine Ophthalmic solution.

- Take some of water for injection of step no 1 in cleaned stainless steel vessels. To it add and dissolve EDTA disodium, octoxynol 40, sodium chloride under continuous stirring to get a clear solution.
- Weigh accurate batch quantity of Ketorolac tromethamine add and dissolve it into the bulk solution of step no 2 under constant stirring to dissolve it completely.
- Measure accurate quantity of benzalkonium chloride and add and dissolve it into the bulk solution of step no 3 to dissolve it completely.
- Check the PH of the resultant bulk solution and if necessary, adjust the ph to 7.4 using 1 N sodium hydroxide solution for 1 N hydrochloric acid solution.
- Make up the volume of the bulk solution to 1.0 lit using water for injection of step no 1.
- Remove the resultant solution (approx) as a before filtration sample and submit it for analysis.
- Filter the remaining bulk solution through 0.22  $\mu$  PVDF membrane filter submit the after filtration sample for analysis.
- Fill the filtered bulk solution into 5 ml 3 piece and 5 ml BFS containers with fill volume of 3 ml.
- Charge the batches for stability at 40°C  $\pm$  2°C/ NMT 25% R H, 25°C  $\pm$  2°C/ NMT 40%  $\pm$  5% R H, 30°C  $\pm$  2°C/ NMT 65%  $\pm$  5% R H, 60°C  $\pm$  2°C.

#### Preservative Efficacy Test (PET) for Ketorolac tromethamine 0.5% ophthalmic solution

**Method:** The method to conduct the experiment should be as per the approved format of the pharmacopoeia.

- Preparation of the stock culture of E. Coli ATCC 8739, Pseudomonas aeruginosa ATCC 9027, Staphylococcus aureus ATCC 6538, Candida albicans ATCC 10231, Aspergillus niger ATCC ATCC 16404 and Anyone of the Environmental isolate Collected from within the premises of the manufacturing facility.
- Preparation of a validated neutralizer solution required for carrying out the experiment.
- Preparation of the plating media used for the experiment to be as per the Pharmacopoeial guidelines.
- The plates which are made during the experiment are to be incubated with the respective temperatures in a sterile environment of an Incubator [8].

#### Procedure for preparation of culture suspension:

##### Preparation of Aspergillus niger culture suspension

- Harvest the culture in 15 ml of sterile saline (0.9% w/v Sodium chloride) containing 0.05% of Polysorbate 80 (Diluent), to obtain a microbial count of  $1 \times 10^7$  to  $1 \times 10^8$  CFU per ml (Stock). Vortex for about 1 minute. From the harvested stock solution, perform the serial dilution up to  $10^{-10}$  by using Diluent.
- From each dilution tube, transfer 1 ml each to a set of sterile petri plate.
- Pour about 20 ml of pre-sterilized Sabouraud dextrose agar at about 45°C.
- Gently rotate the plates in clockwise and anticlockwise direction on LAF bench for uniform mixing of culture and media. Allow the plates to solidify and after solidification of plates, incubate all the plates at 20–25°C for 5 to 7 days [8].

##### Preservation of the diluted culture suspension:

- Store all the diluted culture suspension tubes of step 3.1 in the cooling cabinet (2-8°C) till the final observations are made.
- Record the colony count details of each organism in the format for Culture Stock Preparation [8].

##### Preparation of Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,

##### Candida albicans and Environment isolate:

- Escherichia coli: Subculture in 15 ml of Soyabean casein digest medium, vortex for about 1 minute and incubate at 30 to 35°C for 24 to 48 hrs to get Stock culture suspension.
- Vortex the Stock suspension for 1 minute and transfer 1 ml from stock suspension to a test tube containing 9 ml of sterile 0.1% Peptone marked as  $10^{-1}$ .
- Vortex the  $10^{-1}$  dilution tube and transfer 1 ml each to 2 sterile petri plates and 1 ml to a test tube containing 9 ml of sterile 0.1% Peptone marked as  $10^{-2}$ .
- Continue as given in 8,1,2 further up to the dilution tube  $10^{10}$ .
- Pour about 20 ml of pre-sterilized Soyabean casein digest agar maintained at temperature of about 45°C to each of Petri plate.
- Gently rotate the plates in clockwise and anticlockwise direction on the LAF bench for uniform mixing of culture and media.
- Allow the plates to solidify.
- After solidification of plates, incubate all the plates at 30 to 35°C for 3 days.
- Follow the steps as indicated in 5.1 with Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans and environment isolate, except using Sabouraud dextrose agar and incubation at 20°C to 25°C for 5 days in case of Candida albicans.
- Store all the diluted culture suspension tubes in the cooling cabinet (2-8°C) till the final observations are made.
- Record the colony count details of each organism in the format for Culture Stock Preparation [8].

Sr.no	Concentration of culture in stock culture	Dilution tube to be taken for analysis
1	$1 \times 10^7$ to $10 \times 10^7$ cfu/ml	Stock
2	$1 \times 10^8$ to $10 \times 10^8$ cfu/ml	$10^{-1}$
3	$1 \times 10^9$ to $10 \times 10^9$ cfu/ml	$10^{-2}$
4	$1 \times 10^{10}$ to $10 \times 10^{10}$ cfu/ml	$10^{-3}$

Table 5: Selection of dilution for test.

#### Selection of dilution for test:

- At the end of incubation period, check the plates visually and count the individual plates with the help of colony counter. Record the observations, calculate the average and Enter in the format for Culture Stock preparation [8].
- Take the tube for test containing between  $1 \times 10^7$  to  $9 \times 10^7$  cfu/ml as given in table 5.

#### Procedure for preservative efficacy test [8]

- Take required number of product containers to collect 15 ml of product and transfer the product into the six sterile test tubes.
- Label all the containers with name of Sample, B. No., name of the Organism and Date Inoculation.
- Vortex the selected tube of the respective culture and add 0.15 ml of the selected culture suspension of organisms to the tubes containing 15 ml of sample each.
4. Vortex the contents.

Note: The volume of culture suspension used should be in between 0.5% and 1% of the volume of the product in the container.

- Record the calculation details of quantity of culture suspension required for each organism in format for Sample preparation.
- Carry out the Initial count of the cultures which is added to the Sample as given in 3.1 to 3.6; 5.0 & 6.0 to a dilution up to  $10^{-7}$  and using 9 ml of Sterile Dey-Engley Neutralizing broth as Diluents and document the colony count details in the Report for Initial count. At the end of 6 hours (From the culture addition to the product), carryout the serial dilution of the sample test tube as per illustration below.
- Select the container which is inoculated with *Escherichia coli*, mix the solution by using vortex mixer and transfer 1 ml to 9 ml of sterile neutralizer (Dey-Engley Neutralizing broth. Label the tube with organism name and dilution ( $10^{-1}$ ).
- Vortex the tube ( $10^{-1}$ ) and transfer 1ml each of dilution to a set of sterile Petri dish labeled as  $10^{-1}$  with the name of Sample, B. No. Time interval and Date and to a tube containing 9 ml of sterile neutralizer, Label the tube with organism name and  $10^{-2}$ .
- Follow step number 7.2 for serial dilution up to tube labeled as  $10^{-6}$ .
- Pour about 20 ml of pre-sterilized media of Soya bean casein digest agar to each Petri plate and gently rotate the plates in clockwise and anticlockwise direction on the LAF bench for uniform mixing of culture and media. Allow the plates to solidify.
- After solidification of plates, incubate all the plates at 30 to 35°C for 3 days.

- Repeat the step 7.1 to 7.5 with, *Pseudomonas aeruginosa*, *Staphylococcus aureus* & Environment isolate. Repeat the step 7.1 to 7.5 with *A. niger* & *C. albicans* except using Sabouraud dextrose agar and incubating at 20°C to 25°C for 5 to 7 days.

- Record the colony counts in the format for report for 6 hrs.

- The inoculated sample containers has to be stored at 20 to 25°C up to 28 days for further testing as per schedule time points.

- Repeat the procedure 7.1 to 7.7 for 24 hours, 7<sup>th</sup> day, 14<sup>th</sup> day and 28<sup>th</sup> days and record in the respective time point reports.

- Calculate the log reduction after each time point of test as per formula in report.

#### Acceptance criteria

- For parenteral and ophthalmic preparations was given in table 6.

- The A criteria express the recommended efficacy to be achieved. In justified cases where the A criteria cannot be attained, for example for reasons of an increased risk of adverse reactions, the B criteria must be satisfied.

- Report the results in the Summary Report.

**Storage conditions:** 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. The storage conditions and the length of studies chosen should be sufficient to cover storage, shipment, and subsequent use (Table 7).

**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 8).

**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.

Microorganism		Log reduction					
		6 Hours	24 Hours	7 Day	14 Day	21 Day	28 Day
Bacteria	A	2	3	-	-	-	NR'
	B	-	1	3	-	-	NI''
Fungi	A	-	-	2	-	-	NI''
	B	-	-	-	1	-	NI''

Table 6: Acceptance criteria for Ophthalmic and Parenteral preparation.

Study	Storage condition	Minimum time period covered
Intermediate	30°C ± 2°C/65% RH ± 5% RH	3 Month
Accelerated	40°C ± 2°C/75% RH ± 5% RH	3 Month
Stress condition	60°C ± 2°C	3 Month

Table 7: Storage Condition.

Product	Ketorolac Tromethamine Ophthalmic Solution 0.5% Pack: 3 Piece container and BFS –5 mL						
Condition	Time points	Analytical parameters					Number of samples
		Description assay	Rs	Preservative content	Osmolality and Drop size study	pH	
40°C ± 2°C/ not more than (NMT) 25% RH	INT	(2)	(2)	(2)	(1)	(1)	00
	2 w	(2)	(2)	(2)	(1)	(1)	08
	1 M	(2)	(2)	(2)	(1)	(1)	08
	2 M	(2)	(2)	(2)	(1)	(1)	08
	3 M	(2)	(2)	(2)	(1)	(1)	08
	6 M	(2)	(2)	(2)	(1)	(1)	08
25°C ± 2°C/ 40% ± 5% RH	1 M	(2)	(2)	(2)	(1)	(1)	08
	3 M	(2)	(2)	(2)	(1)	(1)	08
	6 M	(2)	(2)	(2)	(1)	(1)	08
30°C ± 2°C/ 65% ± 5% RH	1 M	(2)	(2)	(2)	(1)	(1)	08
	3 M	(2)	(2)	(2)	(1)	(1)	08
	6 M	(2)	(2)	(2)	(1)	(1)	08
60°C	1 w	(2)	(2)	(2)	(1)	(1)	08
	2 w	(2)	(2)	(2)	(1)	(1)	08
	1 M	(2)	(2)	(2)	(1)	(1)	08
Total							112

Table 8: Stability Protocol.

**Product analysis:** The product analysis was done as per the stability protocol. Finished product tests include Appearance, Assay of ketorolac tromethamine and Preservative content (BKC), pH, Osmolality, 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 Ketorolac tromethamine:** USP recommends high performance liquid chromatography for assay Ketorolac tromethamine of 0.5% ophthalmic solution.

**3. Instrument:** Analytical balance, HPLC, pH meter, ultra sonicator.

**4. Chemical and reagents:** Water, tetrahydrofuran, ammonium dihydrogen phosphate, ortho phosphoric acid, 0.45 μ-nylon membrane filter 47 and 33 mm.

**5. Preparation of buffer:** Weigh about 5.75 g of ammonium dihydrogen phosphate, dissolve in 1000 ml water. Adjust pH to 3.0 ± 0.05 with dilute ortho phosphoric acid. Filter through 0.45 μ-nylon membrane filter, 47 mm.

**6. Preparation of mobile phase:** Mix buffer and tetrahydrofuran in the ratio of 70:30 v/v. sonicates to degas.

**7. Preparation of diluents / blank:** Mix water and tetrahydrofuran in the ratio of 70:30 v/v.

**8. Preparation of standard solution:** Accurately weigh and transfer 40.0 mg of ketorolac tromethamine working standard into 100 ml volumetric flask, add about 75 ml diluents and sonicate to dissolve, dilute up to the mark with diluents and mix well. Filter through 0.45 μ nylon membrane filter 33 mm.

**9. Preparation of test solution:** Accurately weigh 2.0 g (equivalent to 10 mg/ml) of test sample into 25 ml volumetric flask, add about 15 ml diluents and shake well, dilute up to the mark with diluents and mix

well. Filter through 0.45 μ nylon membrane filter 33 mm.

**Procedure of assay of ketorolac tromethamine:** Equilibrate the column with mobile phase for sufficient time (about 30-60 minutes) until stable baseline is obtained. Inject blank, standard and test solution into the chromatograph as per the injection sequence table given below and record the chromatograms, inject the standard solution as a bracketing after every six injection of test solution.

**Calculations:** Calculate the amount of benzalkonium chloride present in mg/ml of solution using following formula:

$$\text{mg/ml} = \frac{AT \times WS \times 5 \times 25}{AS20050WT} \times \text{weight per ml the test sample(1)} \quad (1)$$

Where AT is Average area of Ketorolac tromethamine sample peak in test chromatograms, AS is Average area of Ketorolac peak in standard chromatograms, WS is Weight of Ketorolac tromethamine standard taken in mg, WT is Weight of Ketorolac tromethamine sample taken in grams, P is Potency of Ketorolac tromethamine sample on as is basis.

**Assay of benzalkonium chloride:** USP recommends high performance liquid chromatography for assay Ketorolac tromethamine of 0.5% ophthalmic solution.

- i. **Instrument:** Analytical balance, HPLC, PH meter, ultra sonicator.
- ii. **Chemical and reagents:** Water, acetonitrile, Disodium hydrogen phosphate, ortho phosphoric acid, 0.45 μ-nylon membrane filter 47 and 33 mm.
- iii. **Preparation of buffer:** Weigh about 7.1 g of Disodium hydrogen phosphate, dissolve in 1000 ml water. Adjust ph to 3.0 ± 0.05 with dilute ortho phosphoric acid. Filter through 0.45 μ-nylon membrane filter, 47 mm.
- iv. **Preparation of mobile phase:** Mix buffer and acetonitrile in the ratio of 40:60 v/v. sonicates to degas.
- v. **Preparation of diluents / blank:** Use milli Q water.
- vi. **Preparation of standard solution:** Accurately weigh and transfer 100.0 mg of Benzalkonium chloride working standard

into 200 ml volumetric flask, add about 100 ml diluents and sonicate to dissolve, dilute up to the mark with diluents and mix well. Filter through 0.45 µ nylon membrane filter 33 mm.

- vii. **Preparation of test solution:** Accurately weigh 5.0 g (equivalent to 5 mg/ml) of test sample into 20 ml volumetric flask, add about 10 ml diluents and shake well, dilute up to the mark with diluents and mix well. Filter through 0.45 µ nylon membrane filter 33 mm.

**Procedure of assay of benzalkonium chloride:** Equilibrate the column with mobile phase for sufficient time (about 30-60 minutes) until stable baseline is obtained. Inject blank, standard and test solution into the chromatograph as per the injection sequence table give below and record the chromatograms, inject the standard solution as a bracketing after every six injection of test solution.

Calculate the amount of benzalkonium chloride present in mg/ml of solution using following formula:

$$\text{mg/ml} = \frac{AT \times WS \times 5 \times 20 \times P}{AS \times 200 \times 100 \times WT \times 100} \times \text{weight per ml the test sample} \quad (2)$$

Where *AT* is average area of sum of benzalkonium sample peak 1 peak 2 in test chromatograms, *AS* is average area of sum of benzalkonium sample peak 1 peak 2 in standard chromatograms, *WS* is Weight of s benzalkonium working standard taken in mg, *WT* is Weight of benzalkonium sample taken in grams and *P* is Potency of benzalkonium working standard in % w/w on as is basis.

**pH:** Recalibrated digital pH meter was used for the pH measurement of Ketorolac tromethamine ophthalmic solution.

**Procedure:** The equipment was switched on by pressing the ON/OFF key. Display showed main screen after initial checks. Electrode was rinsed with distilled water and blotted dry with tissue paper. Electrode and temperature probe were dipped in sample solution. By pressing 'MODE' key pH was selected. 'MEAS' was selected to check the pH. After stabilization reading for pH was noted. Again glass electrode was flushed with water and wiped out. It was placed back in bottle congaing buffer solution.

**Osmolality:** Recalibrated digital Osmometer was used for the Osmolarity measurement of Ketorolac tromethamine ophthalmic solution.

**Procedure:** Instrument was switched on and waited till initialization completed. 'START' key was pressed and waited till Running Diagnostic completed. 250 µL of the sample was pipette out in sample tube with help of micro pipette and micro-tip. The probe and stir/freeze wire was cleaned and wiped. 'START' key was pressed and data for sample number was entered. Again 'START' key was pressed. Note the reading

for osmolality in mOsm on digital display. Empty sample tube was leaved in freezing chamber to avoid deposition of debris.

**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.

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

$$\text{Weight of each drop (drop size)} = \text{Weight of 10 drops}/10 \quad (3)$$

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

**Water loss:** Five Semi-permeable containers of each type, LDPE Three piece and BFS, were charged to accelerated condition of temperature and humidity (40°C ± 2°C/ 25% ± 5% RH) with proper labeling on each container. Before charging, each container was weighed individually for initial weights. At every station of stability, each container was individually weighed and noted.

Percentage water loss was calculated by using formula

$$\text{Water loss} = \frac{\text{Initial weight of container} - \text{final weight of container}}{\text{Initial weight of container}} \times 100 \quad (4)$$

## Result and Discussion

### Preservative efficacy test

Log reduction for each type of micro-organism was calculated using the given formula. Calculation of log reduction of microorganism for sample of Ketorolac Tromethamine ophthalmic solution (0.5% w/v) of batch number OPT / KTM/T-001 containing Benzalkonium chloride (0.00 w/v) was done and results of preservative efficacy test for same sample was represented in table 9. As per the above results the given sample of Ketorolac Tromethamine Ophthalmic solution (0.05% w/v) bearing the batch number OPT / KTM/T-001 does not pass the test for Antimicrobial Effectiveness Test as per USP (United state Pharmacopoeia). In the same manner, log reduction calculation of microorganism for various batches of Ketorolac Tromethamine ophthalmic solution (0.5% w/v). Results of Preservative efficacy test for Ketorolac Tromethamine Ophthalmic solution (0.5% w/v) Batch no. OPT / KTM/T-002 containing Benzalkonium chloride (0.5 w/v) was given in table 9.1. As per the above results the given sample of Ketorolac Tromethamine Ophthalmic solution (0.5% w/v) Bearing the Batch no. OPT / KTM/T-002 pass the test for Antimicrobial Effectiveness Test as per USP. Results of Preservative efficacy test for Ketorolac Tromethamine Ophthalmic solution (0.5% w/v) Batch no. OPT / KTM/T-003 containing

Hours/Day	Log reduction			
	Acceptance criteria		Observed results	
	Bacteria and mold	Fungi/Yeast	Bacteria	Fungi/yeast and mold
24 Hours	-	-	-	-
7 <sup>th</sup> day	Not less than 1 Log Reduction	No increase from the Initial count	0.3	No increase
14 <sup>th</sup> day	Not less than 3 Log Reduction	No increase from the Initial count	2	No increase
21 <sup>st</sup> day	-	-	-	-
28 <sup>th</sup> day	No increase from the 14 <sup>th</sup> day count	No increase from the Initial count	No increase	No increase

Table 9: Results of Preservative efficacy test.

Hours/Day	Log reduction			
	Acceptance criteria		Observed results	
	Bacteria and mold	Fungi/yeast	Bacteria	Fungi/yeast and mold
6 Hours	-	-	-	-
24 Hours	-	-	-	-
7 <sup>th</sup> day	Not less than 1 Log Reduction	No increase from the Initial count	4.4	No increase
14 <sup>th</sup> day	Not less than 3 Log Reduction	No increase from the Initial count	4.4	No increase
21 <sup>st</sup> day	-	-	-	-
28 <sup>th</sup> day	No increase from the 14 <sup>th</sup> day count	No increase from the Initial count	No increase	No increase

**Table 9.1:** Preservative efficacy test [Preservative added: Benzalkonium chloride (0.05 w/v)].

Hours/Day	Log reduction			
	Acceptance criteria		Observed results	
	Bacteria and mold	Fungi/yeast	Bacteria	Fungi/yeast and mold
6 Hours	-	-	-	-
24 Hours	-	-	-	-
7 <sup>th</sup> day	Not less than 1 Log Reduction	No increase from the Initial count	4.4	No increase
14 <sup>th</sup> day	Not less than 3 Log Reduction	No increase from the Initial count	4.4	No increase
21 <sup>st</sup> day	-	-	-	-
28 <sup>th</sup> day	No increase from the 14 <sup>th</sup> day count	No increase from the Initial count	No increase	No increase

**Table 9.2:** Result of Preservative efficacy test [Preservative added: Benzalkonium chloride (0.06 w/v)].

Hours/Day	Log reduction			
	Acceptance criteria		Observed results	
	Bacteria and mold	Fungi/yeast	Bacteria	Fungi/yeast and mold
6 Hours	-	-	-	-
24 Hours	-	-	-	-
7 <sup>th</sup> day	Not less than 1 Log Reduction	No increase from the Initial count	4.5	No increase
14 <sup>th</sup> day	Not less than 3 Log Reduction	No increase from the Initial count	4.5	No increase
21 <sup>st</sup> day	-	-	-	-
28 <sup>th</sup> day	No increase from the 14 <sup>th</sup> day count	No increase from the Initial count	No increase	No increase

**Table 9.3:** Results of Preservative efficacy test [Preservative added-Benzalkonium chloride (0.08 w/v)].

Hours/Day	Log reduction			
	Acceptance criteria		Observed results	
	Bacteria and mold	Fungi/yeast	Bacteria	Fungi/yeast and mold
6 Hours	-	-	-	-
24 Hours	-	-	-	-
7 <sup>th</sup> day	Not less than 1 Log Reduction	No increase from the Initial count	4.5	No increase
14 <sup>th</sup> day	Not less than 3 Log Reduction	No increase from the Initial count	4.6	No increase
21 <sup>st</sup> day	-	-	-	-
28 <sup>th</sup> day	No increase from the 14 <sup>th</sup> day count	No increase from the Initial count	No increase	No increase

**Table 9.4:** Results of Preservative efficacy test [Preservative added-Benzalkonium chloride (0.10 w/v)].

Hours/Day	Log reduction			
	Acceptance criteria		Observed results	
	Bacteria and mold	Fungi/yeast	Bacteria	Fungi/yeast and mold
6 Hours	-	-	-	-
24 Hours	-	-	-	-
7 <sup>th</sup> day	Not less than 1 Log Reduction	No increase from the Initial count	4.6	No increase
14 <sup>th</sup> day	Not less than 3 Log Reduction	No increase from the Initial count	4.6	No increase
21 <sup>st</sup> day	-	-	-	-
28 <sup>th</sup> day	No increase from the 14 <sup>th</sup> day count	No increase from the Initial count	No increase	No increase

**Table 9.5:** Result of Preservative efficacy test [Preservative added- Benzalkonium chloride (0.12 w/v)].

benzalkonium chloride (0.06 w/v) was given in table 9.2. As per the above results the given sample of Ketorolac Tromethamine Ophthalmic solution (0.5% w/v) bearing the batch no OPT / KTM/T-003 pass the test for Antimicrobial Effectiveness Test as per USP.

Results of preservative efficacy test for Ketorolac Tromethamine

Ophthalmic solution (0.5% w/v) batch no OPT / KTM/T-004 containing benzalkonium chloride (0.08 w/v) was given in table 9.3. As per the above results the given sample of Ketorolac Tromethamine Ophthalmic solution (0.5% w/v) bearing the batch no OPT / KTM/T-004 pass the test for Antimicrobial Effectiveness Test as per USP. Results of preservative efficacy test for Ketorolac Tromethamine Ophthalmic solution (0.5%

w/v) batch no OPT / KTM/T-005 containing benzalkonium chloride (0.10 w/v) was given in table 9.4. As per the above results the given sample of Ketorolac Tromethamine Ophthalmic solution (0.5% w/v) bearing the batch no OPT / KTM/T-005 pass the test for Antimicrobial Effectiveness Test as per USP. Results of Preservative efficacy test for Ketorolac Tromethamine Ophthalmic solution (0.5% w/v) batch no OPT / KTM/T-006 containing benzalkonium chloride (0.12 w/v) was given in table 9.5. As per the above results the given sample of Ketorolac Tromethamine Ophthalmic solution (0.5% w/v) bearing the Batch no. OPT / KTM/T-006 pass the test for Antimicrobial Effectiveness Test as per USP. As summarizing the results of Preservative efficacy test, results for Bacterial log reduction with different concentration of

BAK Concentration	Observation (Log reduction)					
	6 HRS	24 HRS	7 DAY	14 DAY	21 DAY	28 DAY
0.0%	0	0	0.3	2	0	0
0.05%	0	0	4.4	4.4	0	0
0.06%	0	0	4.4	4.4	0	0
0.08%	0	0	4.5	4.5	0	0
0.10%	0	0	4.5	4.6	0	0
0.12%	0	0	4.6	4.6	0	0
Acceptance criteria	-	-	Min 1 Log Reduction	Min 3 Log Reduction	-	No Recovery

Table 10: Log reduction in Bacterial growth.

BAK Concentration	Observation (Log reduction)					
	6 HRS	24 HRS	7 DAY	14 DAY	21 DAY	28 DAY
0.0%	0	0	0	0	0	0
0.05%	0	0	0	0	0	0
0.06%	0	0	0	0	0	0
0.08%	0	0	0	0	0	0
0.10%	0	0	0	0	0	0
0.12%	0	0	0	0	0	0
Acceptance criteria	-	-	No increase	No increase	-	No increase

Table 11: Log reduction in Fungal growth.

Sr. No.	Result	T01	T02	T03	T04	T05	T06
1	ASSAY	96.3	102.5	96.4	99.4	99.5	100.5
2	BKC	0	29.2	34.3	53	62.7	67.1
3	PH	7.4	7.4	7.4	7.4	7.4	7.4

Table 12: Assay of Ketorolac tromethamine bkc result.

Sr.No.	Result	T07	T08	T09	T10
1	ASSAY	99.8	98.5	95.5	96.7
2	BKC	96	96	92.3	94.5

Table 13: Concentration Change with NaCl.

Product: Ketorolac tromethamine Ophthalmic solution												
Strength: Ophthalmic solution (0.5% w/v)						Batch no: OPT/KTM/T-001						
Description												
Clear colorless liquid filled in BFS Container												
Stability Condition		40°C/NMT 25% RH			30°C/ 65% RH			25°C/ 40% RH				
Test parameters		Initial AF	Initial BF	1 M	2 M	3 M	1 M	2 M	3 M	1 M	2 M	3 M
Description												
Assay %		99.7	99.2	98.5	98.2	98.2	98.6	98.4	98.6	98.4	98.4	98.6
Preservative Content in %		87.6	90	86.4	86.9	89.3	91.6	90.9	91.6	91.6	92.6	92.8
Osmolality		NA	295	295	299	285	300	302	288	301	306	301

Table 14: Product result.

benzalkonium chloride are summarized in following table 10 and results for Fungal log reduction with different concentration of Benzalkonium chloride are summarized in following table 11. Over all experimental conclusion with repeat to antimicrobial preservative test as per USP for Ketorolac tromethamine ophthalmic solution (0.5% w/v) Ketorolac tromethamine ophthalmic solution (0.5% w/v) containing (0.05% w/v) benzalkonium chloride passes the test as per USP.

### Results of stability/containter compatibility study

In stability/container compatibility study drug product was evaluated for assay of Ketorolac tromethamine 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. Results for Ketorolac tromethamine assay are depicted in the table 12. The batch OPT/ KTM/01 TO 06 this formulation was taken without BKC concentration, as it is conclude that assay of Ketorolac tromethamine was not possible. The result of this study shows that no significant effects were observed on turbidity and fungal contamination of not growth micro-organism.

### Results for concentration change with NaCl

The batch OPT/KTM/TO7 TO 10 this formulation was taken the different concentration of NaCl. As it is concluded that as per above, batch no 08 had stable isotonicity and reduce eye irritation (Table 13).

### pH observations

The change in the pH of the formulation during stability testing can be indicative of either degradation of the active ingredient or interaction of one more of the constituent of the formulation with the container. The pH of the solution should be in between 7.2 to 7.8, as per the specification. The pH of initial sample was 7.40. The pH of the solution charged for stability at various conditions and time interval was found to be in between 7.2 to 7.8 (Table 14).

### Assay of ketorolac tromethamine

The ketorolac tormethamine was evaluated at initial, at stress condition and at accelerated condition. Initially assay of Ketorolac tromethamine was found to be B.F 99.7 & A.F 99.2. At accelerated condition of temperature and relative humidity (40°C ± 2°C/ NMT 25% R H for plastic and 40°C ± 2°C/ NMT 75% RH) assay was estimated up to three months (Table 14). Assay found to Initial before filtration 99.7, Initial after filtration 99.2 40°C/NMT 25% RH 1 m 98.5, 2 m 98.2, 3 m 98.2 0°C/ 65% RH 1 m 98.6, 2 m 98.4, 3 m 98.6. 25°C/ 40% RH 1 m 98.4, 2 m 98.4, 3 m 98.6 (Table 14). Loss of Ketorolac tromethamine 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 Ketorolac tromethamine to long polymeric chain of carbon present in MOC. Another possible reason is

Sr. No.	Containers	Initial	Stress condition		Accelerated condition		
			1 Week	2 Week	1 Month	2 Month	3 Month
1	Three piece	297	290	294	290	298	289
2	BFS	295	297	292	290	292	293

Table 15: Osmolarity result.

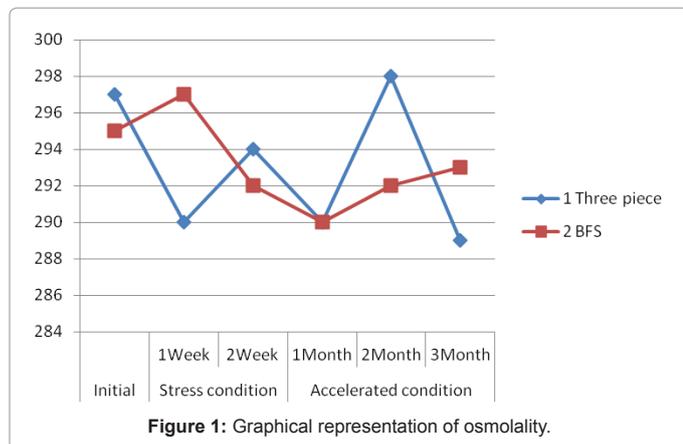


Figure 1: Graphical representation of osmolarity.

that entrapment of Ketorolac tromethamine 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.

### Assay of benzalkonium chloride

Similar to assay of Ketorolac tromethamine, 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 B.F 87.6 & A.F 90.0 (Table 14). At accelerated condition of temperature and relative humidity (40°C ± 2°C/ NMT 25% R H for plastic and 40°C ± 2°C/ 75 ± 5% RH) assay was estimated up to three months. Significant loss in assay was found 40°C/NMT 25% RH 1 m 86.4, 2 m 86.8, 3 m 98.2, 30°C/ 65% RH 1 m 98.6, 2 m 98.4, 3 m 98.6. 25°C/ 40% RH 1 m 98.4, 2 m 98.4 3 m 98.6 (Table 14). Benzalkonium chloride has the tendency for adsorption on to the surface of plastic. Loss of preservative may be due to chemical adsorption of C<sup>12</sup> - C<sup>18</sup> 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.

### 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 leachable that may be added to solution from the wall of container. The results for the osmolality are tabulated in table 15. 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 315 mOsm (initial) to 338 mOsm at 3 month (Figure 1). In BFS containers it was increased to 357 mOsm and in glass container it was increased to 348 mOsm. At stress condition osmolality in BFS container is more than the osmolality at accelerated condition (Figure 1). 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. Ofloxacin ophthalmic solution formulation contains hydrochloric acid. This hydrochloric acid may attack on cross linkage of polymer. This acidic 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 AUW-220, Shimadzu balance. The results obtained are tabulated in table 16. From the above results it has been found that up to third month in accelerated condition drop size of BFS containers (40.72 µL) was comparatively more increased than three piece container (35.03 µL) (Figure 2). 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 (Figure 2). In case of three pieces container nozzles are made up of same polymer used for body but contain additional ingredients which increase the rigidity of nozzle (Figure 2). Thus at accelerated condition of stability softening and

Sr. No.	Containers	Initial	Accelerated condition		
			1 Month	2 Month	3 Month
1	Three piece	33.40	33.93	35.03	36.18
2	BFS	34.21	36.85	40.68	40.72

Table 16: Drop size of three piece and BFS Container Observations.

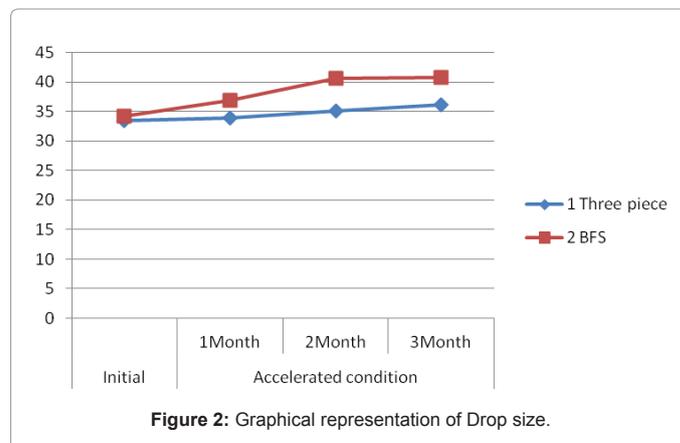


Figure 2: Graphical representation of Drop size.

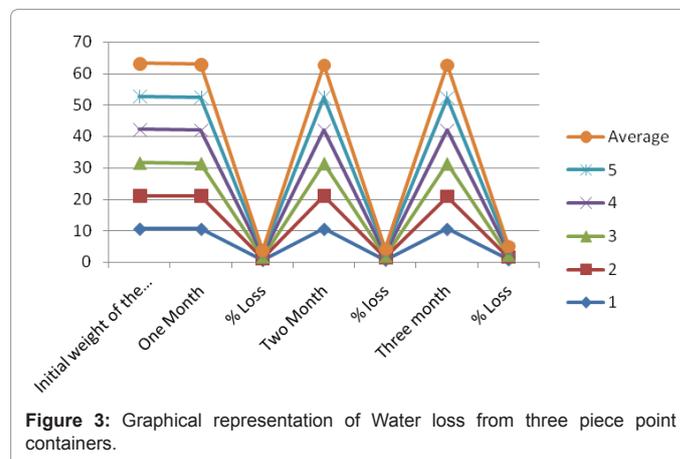


Figure 3: Graphical representation of Water loss from three piece point containers.

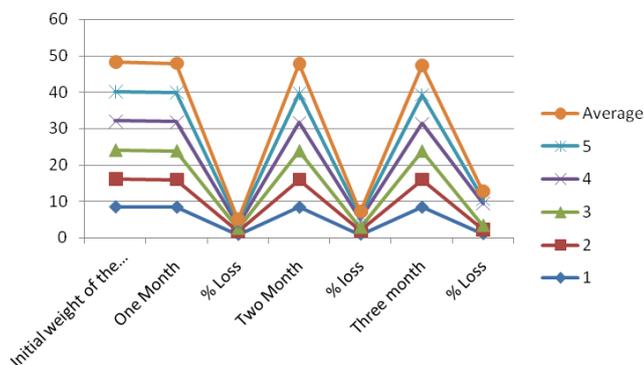


Figure 4: Graphical representation of water loss from BFS container.

Container no.	Initial weight of the container(g)	One month	% Loss	Two month	% Loss	Three month	% Loss
1	10.539	10.4796	0.5636	10.4707	0.6481	10.4562	0.7857
2	10.5321	10.47	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	10.558	0.8443	10.5428	0.987
5	10.4737	10.4016	0.6883	10.3929	0.7715	10.3808	0.887
Average	10.5376	10.4744	0.5992	10.4652	0.6859	10.452	0.8119

Table 17: Water loss study- Three piece containers.

Container no.	Initial weight of the container(g)	One month	% Loss	Two month	% Loss	Three month	% 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	7.868	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	8.041	7.9748	0.8223	7.9452	1.1904	7.8716	2.1043

Table 18: Water loss study- BFS container.

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°C/25% RH. 5 containers of each type i.e. 3 piece and BFS were placed in upright position for water loss. The results obtained are presented in tables 17 and 18, figures 3 and 4. 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 (Table 17) where as for BFS containers water loss was 0.8223%, 1.1904%, and 2.1043% respectively (Table 18) though the both type of containers passed the criteria of water loss i.e. not more than 5.0% (Figures 3 and 4). BFS containers are showing the more water loss up to 2.1043% (Table 18) and three piece containers are showing water loss just up to 0.8119% (Table 17). Environmental stress cracking resistance (ESCR) number of three piece container (PE 1840 H) is higher than ESCR number of BFS (PE 3020 D) container (Figures 3 and 4). 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.

### Conclusion

In this investigation the concentration of preservative benzalkonium chloride vary from 0.05 w/v to 0.12 w/v. The Preservative Efficacy test was done and all batches except batch no OPT/KTM/T-001 not passes test because does not contain preservative. The least concentration of preservative benzalkonium chloride i.e., 0.05 w/v in batch no OPT/KTM/T-002 passes the preservative test. Hence this batch was optimized. From above study least effective concentration of preservative Benzalkonium chloride was established. The present research work provide the data about the selection of suitable primary packaging material for Ketorolac tromethmine (0.5%) to achieve the better stability during the shelf life of the product. olution.

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