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Original Article

Design and Evaluation of Ophthalmic Delivery of Bepotastine Besilate From Eye Drop

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ABSTRACT

Introduction: The purpose of present study was to design and evaluate bepotastine besilate ophthalmic solution 1.5% to develop a stable formulation using buffering agent, tonicity modifier and preservative. **Method:** In this study the concentration of preservative and tonicity modifier concentration are adjusted in such a way so that the final formulation will have least concentration of preservative still it will be protected from microorganisms and isotonic so that after instillation when formulation will come in contact with tissues there should not be any swelling, contraction or discomfort. **Result:** The preservative content was selected in the range of 0.002–0.012% and tonicity modifier in the range of 0.45–0.9%. Bepotastine besilate ophthalmic solution 1.5% filled in LDPE container having fill volume 5 mL is clear, colourless solution having pH range 6.8–7.0, drop size of 38.6 µl and % water loss after 3 months as 1.9748%. The percent purity of bepotastine besilate and preservative was determined using RP-HPLC method was found to be 100.50 and 51.648% with % RSD as 0.84 and 0.65% respectively. The samples of the formulation also subjected to stability as per ICH guidelines and 6 months data was generated. Draize test protocol was also followed to study invivo eye irritancy of the formulation to produce a safe and effective formulation. **Conclusion:** Laboratory prepared formulation of bepotastine besilate ophthalmic solution 1.5% has showed good stability at both 25°C and 40°C as the drug and preservative content was within the accepted range.

Key words: Bepotastine besilate, Benzalkonium chloride eye drop, Preservative, Ophthalmic preparation, Stability study, Isotonicity.

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INTRODUCTION

Conjunctivitis refers to inflammation of the conjunctiva, and allergic conjunctivitis occurs when this is caused by an allergic reaction. This is most commonly a type 1 hypersensitivity reaction and it gives rise to seasonal or perennial allergic conjunctivitis. The treatment is based on the administration of eye drops and eye ointment. Bepotastine is a piperidine derivative that antagonizes H1 receptors with high selectivity. It has been labelled a dual acting or multiple-acting anti allergic medication, because it inhibits histamine at H1 receptors and stabilizes mast cells to prevent histamine release. Bepotastine may also have other immune active properties, such as inhibition of eosinophil migration, interleukin-5 (IL-5), leukotrienes (e.g. LTB4) and platelet-activating factor (PAF).[1,2]

Bepotastine besilate (Bepo B) chemically known as ({d-(S)-4-[4-[(4-chlorophenyl) (2-pyridyl) methoxy] piperidino} butyric acid monobenzene sulphonate), is a new second-generation antihistamine developed in Japan. It reduces the natural chemical histamine in the body which can produce allergic symptoms of itching or watery eyes. The chemical structure of bepotastine besilate is depicted in Figure 1.

Benzalkonium chloride (BKC), a typical quaternary ammonium salt, is often used as an antiseptic. Its structure is shown in Figure 2, and the C12 homolog is the major species in a benzalkonium chloride preparation.[3] The mode of antiseptic action of quaternary ammonium compounds appears to be associated with their effect on the cytoplasmic membrane that controls cell permeability, and the C12 homolog is most effective against yeast and fungi.[4] Benzalkonium chloride (BAK) belongs to Quaternary ammonium compound class used as preservative in ophthalmic solution. Kill microorganisms by disrupting cell membranes and causing cell lyses. It should 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.[5]

Delivery of medication to the human eye is an integral part of medical treatment.[6] 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.[7]

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.[8]

In the present research work formulation optimization and evaluation is done in terms of description, pH, assay of bepotastine besilate, assay of benzalkonium chloride, viscosity, osmolarity, drop size study, water loss study, stability study which includes accelerated, long term, freeze thaw and light. Preservative efficacy study, sterility test and invivo eye

irritancy test are also the important parameters which are evaluated.

MATERIALS AND METHOD

Materials

API (Bepotastine besilate-100.4% purity) was supplied by Bal Pharma Ltd. Bommasandra Industrial area, Bangalore, India. Bepotastine besilate ophthalmic solution 1.5% is available under the brand name Bepreve™ by ISTA Pharmaceuticals, Inc. Irvine, CA. Methanol, Acetonitrile (HPLC grade) was obtained from Finar Limited, Ahmadabad, India. Furthermore, Triethylamine (HPLC grade) procured from Himedia Laboratories, Mumbai, India. Potassium dihydrogen phosphate, sodium hydroxide, hydrochloric acid and hydrogen peroxide (AR grade) obtained from Qualigens Fine Chemicals, Mumbai, India. All the inactive ingredients selected were of AR grade.

Methods

Formulation studies: Development of bepotastine besilate ophthalmic solution was done in two phase. Firstly Prototype formulation was developed and then designs the final formula for manufacturing of bepotastine besilate ophthalmic solution.

Prototype formulation development: The prototype formulation for bepotastine besilate was developed using suitable inactive ingredients. The levels of inactive ingredients used in the bepotastine besilate ophthalmic solution, 1.5% confirm to the reported eIIG levels of Inactive ingredients database[9] are shown in Table 1.

Formulation design: The formula for bepotastine besilate ophthalmic solution was designed and optimized. The optimization of proposed formula was done by varying the concentration of Benzalkonium chloride. The quantities of other excipients were kept constant. As the aim of the present study was to optimize the concentration of Benzalkonium chloride in formulation for bepotastine besilate (1.5%) ophthalmic solution. Batches were also planned by taking different concentrations of Benzalkonium chloride in the range of 0.002% to 0.01% and also ophthalmic formulation without preservative and after evaluating the results of preservative efficacy study the final concentration benzalkonium chloride was decided.

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 2).

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[10] (Table 3).

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.

Product analysis: The product analysis was done as per the stability protocol. Finished product tests include Appearance, Assay of bepotastine besilate 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.

Appearance: Sample under test was inspected visually for color and clarity

pH: Recalibrated digital pH meter was used for the pH measurement of bepotastine besilate ophthalmic solution.

Isotonicity: Isotonicity is important characteristic of the ophthalmic formulation.[11] Isotonicity has to be maintained to prevent tissue damage or irritation of eye (Figure 3). Prototype formulations were subjected to isotonicity testing. . Formulations were mixed with few drops of blood and observed under microscope at 45X magnification and compared with standard 0.9% NaCl solution which depicts the isotonic nature.

The shape of blood cell was compared with standard 0.9% NaCl solution.

Drop size: Drop size of instilled drop is the function of the amount of drug delivered to the eye per instillation. This study was performed to evaluate and confirm that Ophthalmic Solution under test has drop size sufficient to deliver the therapeutically effective amount of drug and is comparable with Innovator.

Average drop size of many commercially available topical medication is actually 39 µL with range of 25.1 µL to 56.4 µL.

Randomly three product bottles that are plugged and capped properly were chosen. Measuring cylinder was kept on the balance and tared. The bottles were decapped. The bottle were inverted at 45° angle and finger pressure was applied so as to deliver a drop into the measuring cylinder, weight of the drop was noted down and balance reading was tared to zero. The step was repeated till solution in the measuring cylinder is filled up to 1 mL.

The average weight of single drop, number of drop required for making volume 1 mL, weight per mL (density) of the solution were calculated. The drop size was calculated as follows: -

Drop size in µL = Average weight of drop (mg) / weight (g) per mL of solution under test.

Water loss

Water loss study is performed to evaluate and confirm that Ophthalmic Solution under test in selected container closure system, container size, and fill, would demonstrate a linear water loss at the alternative relative humidity over the storage period, as required by the International Conference on Harmonization (ICH) guidelines (ICH Q1A(R2) Stability Testing of New Drug Substances and Products).

Thirty bottles filled with exact volume of sample solution to be tested, plugged and capped properly were selected. Ensure that only the bottle cap should be labelled so that body of the containers would be properly exposed to selected storage condition. The initial weight of each individual container was noted. The exact weight of each individual container after three months stored at above-mentioned condition was estimated. The average total weight loss of the container was calculated. Percent water loss from the data obtained was calculated as follows:-

Water loss, % =(Average initial weight–Average final weight) / density of solution x 100 / fill volume

Assay of bepotastine besilate ophthalmic solution 1.5%: RP-HPLC method was developed for simultaneous estimation of bepotastine besilate and benzalkonium chloride.

Chromatographic condition and equipment

The HPLC system consisted of Shimadzu HPLC 1100 series consisted of binary pump

LC-10 ADvp, Rheodyne universal injector 7725i and Shimadzu SPD-10 UV–Visible detector. The chromatographic separations were per-

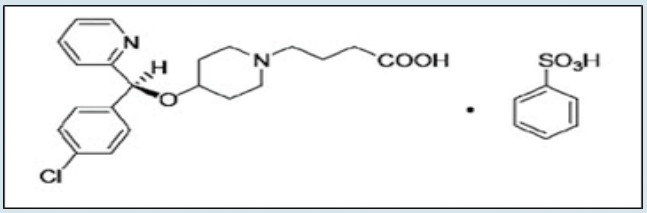

Figure 1: Structure of Bepotastine Besilate.

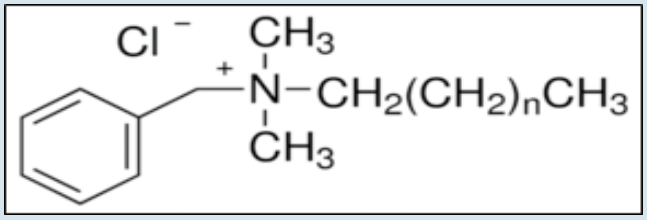

Figure 2: Structure of Benzalkonium chloride.

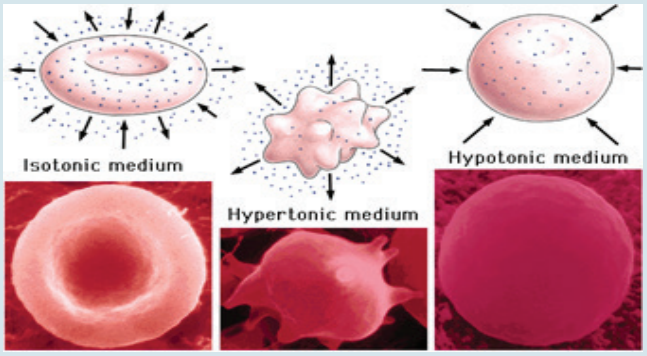

Figure 3: Comparison of Isotonic, hypertonic and hypotonic blood cells.

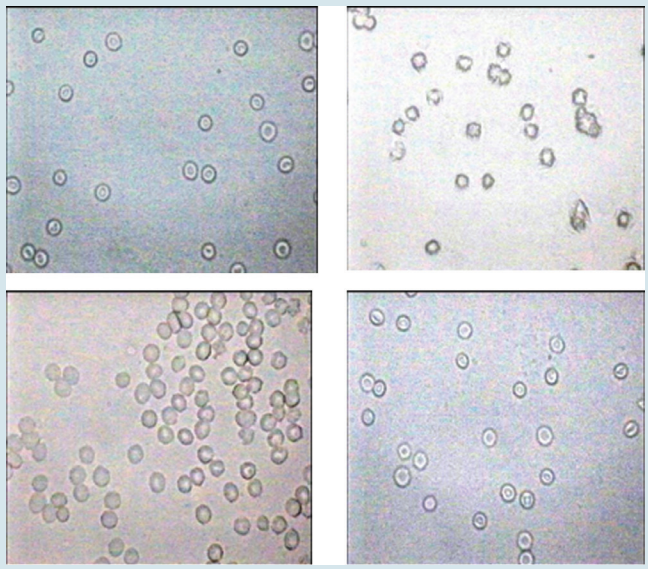

Figure 4: Blood cells in a) Isotonic b) Hypertonic c) hypotonic formulation of Bepotastine besilate ophthalmic solution and d) 0.9% NaCl solution.

formed using Analytical® Hyperchrome ODS C18, 5 µm, 250×4.6 mm i.d. column, at ambient temperature, eluted with mobile phase at the flow rate of 1.0 mL/min. The mobile phase consisted of acetonitrile and potassium dihydrogen phosphate buffer (60:40, v/v), apparent pH adjusted to 5.5 ± 0.1 with phosphoric acid solution, filtered through 0.45 µm nylon filter and degassed in ultrasonic bath prior to use. Wavelength was selected by scanning standard solutions of both drugs over 200 to 400 nm wavelengths using Shimadzu model 1601 double beam UV–visible spectrophotometer with a pair of 10 mm matched quartz cells. Measurements were made with injection volume 20 µL and ultraviolet (UV) detection at 210 nm, as both components show a reasonable good response at this wavelength.[12]

Photostability study

The objective of this study is to evaluate and confirm that Ophthalmic Solution under test contained in the secondary package, will withstand exposure to light when stored under controlled room temperature storage conditions, as required by the International Conference on Harmonization (ICH) guidelines (ICH Q1B Photo stability Testing of New Drug Substances & Products).

The sample (tested and control) will be stored upright exposed to the light source for the predetermined number of days / hours. The distance between the light source and the container surface will be kept constant to achieve uniform radiance. Samples will be exposed to light, providing an overall illumination of not less than 1.2 million lux (average foot candles x 10 x24 hrs / day x total days), and an integral near ultraviolet energy of not less than 200 watts hours/square meter, (milliwatts/cm^2 X10X24 hrs / day x total days), as required by ICH. Actual light intensity and exposure time will be documented and the total illumination will be calculated (Table 4).

Freeze thaw study: This study was performed to determine the effects of temperature variations on the ophthalmic solution under test upon freeze-thaw cycling so as to confirm that the final product is stable during shipping.[13]

20 LDPE bottles filled with ophthalmic solution stored at room temperature were selected and placed in freezer. The complete freezing should be achieved within first 24 h. If not, the bottle was shaken to facilitate the freezing process.

Cycle 1: The samples were kept at–20°C ± 5°C for 48 h and ensure that samples should be completely frozen. All the bottles were removed from freezer and placed in 30°C storage chamber for 48 hrs. After 48 h the bottles were removed from the chamber and the observations were noted down. Similar three cycles were repeated and after third cycle samples were analysed.

Preservative efficacy study

The method to conduct the experiment should be as per the approved format of the Indian pharmacopoeia.

Any antimicrobial agent may show the protective properties of preservative. However, for the protection of the consumer the concentration of the preservative shown to be effective in final packaged product should be considerably below the concentration of the preservative that may be toxic to human beings. The test organisms used in the study are candida albicans ATCC 10231, Aspergillus niger ATCC 16404, Eschrichia coli ATCC 8739, Pseudomonas aeuriginosa

ATCC 9027, Staphylococcus aureus ATCC 6538. The inoculums of the above microorganisms are grown on the surface of suitable agar plate from a recently grown stock culture. The cells were harvested using the solution and suspended to result in a microbial count of about "100 million microorganisms per ml".

Bepotastine besilate ophthalmic solution 1.5% was transferred to five

Table 1: Prototype Formulation

Sr. No.	Category	Maximum potency as per eIIG (%)
1	Preservative	0.51
2	Buffering Agent	1.053
3	Tonicity modifier	0.9
4	Alkali	0.1
5	Acid	0.1

Table 2: Storage condition

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 3: Stability Protocol

Product	Bepotastine besilate ophthalmic solution 1.5%			Pack: 3 piece LDPE container		
Condition	Time points	Analytical parameters				Number of samples
		Description Assay	Preservative content	Osmolality and drop size study	pH	
40°C ± 2°C/ not more than (NMT) 25% RH	INT	2	2	2	2	8
	2W	2	2	2	2	8
	1M	2	2	2	2	8
	2M	2	2	2	2	8
	3M	2	2	2	2	8
	6M	2	2	2	2	8
25°C ± 2°C/ 40% ± 5% RH	1M	2	2	2	2	8
	3M	2	2	2	2	8
	6M	2	2	2	2	8
60°C	1W	2	2	2	2	8
	2W	2	2	2	2	8
	1M	2	2	2	2	8
Photostability Study	Positive control	2	2	2	2	8
	Test control	2	2	2	2	8
	Dark control	2	2	2	2	8
Freeze thaw Study	Initial	2	2	2	2	8
	At the end of 3rd cycle	2	2	2	2	8
Total						136

Table 4: Sample Description for Photostability study in light chamber at 25°C ± 2°C / ambient humidity

Sample description	Packaging information
Test control	Individual covered packaging cardboard
Positive control	Immediate pack (naked LDPE bottles)
Dark control	Bottles with secondary pack covered in Aluminium foil

Table 5: Results of preservative efficacy study

Sr.No.	Conc . of BKC(%)	S. Aureus	E.Coli	P.Auriginosa	A.niger	C. albicans
1	0.002	+	+	+	+	+
2	0.004	+	+	+	+	+
3	0.005	-	-	-	-	-
4	0.006	-	-	-	-	-
5	0.008	-	-	-	-	-
6	0.01	-	-	-	-	-
7	0.00	+	+	+	+	+

= Presence of clear solution (Inhibition), + = presence of turbidity (no inhibition)

Table 6: Stability data

Product		Bepotastine besilate ophthalmic solution 1.5%				
Condition	Time points	Analytical parameters				
		Drug Assay %	% Preservative content	Isotonicity	Appearance	pH
40°C ± 2°C/ not more than (NMT) 25% RH	INT	100.50	51.64	+	CCS	6.80
	2W	96.14	50.89	+	CCS	6.92
	1M	98.93	52.76	+	CCS	7.01
	2M	98.32	50.53	+	CCS	6.83
	3M	96.73	48.32	+	CCS	6.90
	6M	97.89	46.87	+	CCS	6.94
25°C ± 2°C/ 40% ± 5% RH	1M	97.95	51.42	+	CCS	6.81
	3M	98.45	44.26	+	CCS	6.89
	6M	96.23	44.86	+	CCS	6.93
60°C	1W	101.62	40.04	+	CCS	6.98
	2W	93.82	41.36	+	CCS	6.84
	1M	95.16	41.72	+	CCS	6.82
Photostability Study	Positive control	98.12	48.57	+	CCS	6.91
	Test control	97.34	49.08	+	CCS	6.82
	Dark control	100.06	53.49	+	CCS	6.93
Freeze thaw Study	Initial	100.50	51.62	+	CCS	6.80
	At the end of 3rd cycle	97.89	48.96	+	CCS	6.91

+ = Isotonic, CCS= Clear colorless solution, M= Month, W= Week, INT= Initial

Table 7: Drop size of LDPE container

Container	Initial	Accelerated condition		
		1 Month	2 Month	3 Month
LDPE bottles	36.8 µl	36.2 µl	35.9 µl	36.2 µl

tubes of 20 mL each inoculated with 0.1 mL of appropriate microbial stock (inoculums at a concentration of approximately 50 million CFU per ml) These tubes went to be held at 30–32°C during the test. Incubate the inoculated containers or tubes at 20 to 25°C. Determine the viable count by turbidity method at 7, 14, 21 and 28 days subsequent to inoculation. Record also any change observed in the appearance.

In vivo eye irritancy test

The Draize technique was designed for the ocular irritation potential of the ophthalmic product prior to marketing. According to the Draize test, the amount of substance applied to the eye is normally 100 µL placed into the lower cul-de-sac with observation of the various criteria made at a designed required time interval of 1 h, 24 h, 48 h, 72 h, and 1week after administration. Four rabbits (male) weighing 1.5 to 2 kg were used for

Table 8: Eye irritation testing: Rabbit corneal observations for opacity and area of cornea involved

Opacity	Normal rating for opacity	Rating for formulation	Area of cornea involved	Normal rating for corneal area involved	Rating for formulation
No opacity	0 none	0	25% or less not zero	1	0
Diffuse area, details of iris clearly visible	1 slight	0	25% to 50%	2	0
Easily visible transulescent areas, details of iris slightly obscure	2 mild	0	50% to 75%	3	0
Opalescnent areas, no details of iris	3 moderate	0	Greater than 75%	4	0
Opaque, iris Invisible	4 severe	0	-	-	0

Table 9: Rabbit conjunctiva observation

Redness Normal	Rating	Rating for Formulation
Vessels normal	0	0
Vessels definitely injected above Normal	1 slight	0
More diffuse, deeper crimson red with individual vessels not easily discemible	2 moderate	0
Diffuse beefy red	3 severe	0

Table 10: Rabbit iris observations

Values	Normal Rating	Rating for formulation
Normal	0 None	0
Folds above normal, congestion, swelling, iris react to light	1 slight	0
No reaction to light, haemorrhage, gross destruction	2 severe	0

the present study. The sterile formulation was instilled twice a day for a period of 7 days, and a cross-over study was carried out (a 3 day washing period with saline was carried out before the cross-over study). Rabbits were observed periodically for redness, swelling, watering of the eye.

RESULT AND DISCUSSION

Appearance

The laboratory prepared formulation of bepotastine besilate ophthalmic solution 1.5% was found to be a clear colourless solution (CCS).

pH

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 6.8 to 7.0, as per the specification. The pH of initial sample was 6.8. The pH of the solution charged for stability at various conditions and time interval was found to be in between 7.2 to 7.0.

Isotonicity

Tonicity is a measure of the osmotic pressure of two solutions separated by a semi-permeable membrane. Ophthalmic solutions should be isotonic with the body fluids so that when they will come in contact with tissues there should not be any swelling, contraction or discomfort. So to formulate an isotonic preparation concentration of sodium chloride was optimized considering 0.9% NaCl solution as the standard one. The figures depicted below shows the formulations which were isotonic, hypertonic and hypotonic.

The isotonicity of the formulation was evaluated by comparing the blood cells in 0.9% NaCl solution (Figure 4a and 4d), considered as isotonic solution. From the study isotonic formulation was selected and further evaluated.

Preservative efficacy study

Preservative efficacy study shows that ophthalmic formulation is effective in the range of 0.005-0.01% for all the selected microorganisms. The final concentration of preservative selected is in the range of 0.005–0.01% (Table 5).

Assay of drug and Preservative

Assay for Bepo B and BKC was found to be 100.50 and 51.648% with % RSD as 0.84 and 0.65% respectively. The mean % recovery for bepotastine and benzalkonium chloride was found to be 100.09 and 100.81% respectively and % RSD was found to be 0.21 and 0.85% respectively which meets the established acceptance criteria.

The results of stability study of bepotastine besilate ophthalmic solution are shown in Table 6.

Drop size study

The drop size was estimated on average weight basis of drops of ophthalmic solution by using the AUX-220, Shimadzu balance.

The results obtained are tabulated in Table 7.

Increase in drop size may be caused due to widening of the nozzle aperture or thinning of the solution at accelerated condition which was not observed in optimized formulation. In the present study no widening of nozzle aperture as there is no drastic change in drop size.

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. 30 containers of LDPE were placed in upright position for water loss. % water loss for bepotastine besilate ophthalmic solution was found to be 1.9748% which passes the criteria for water loss i.e. not more than 5%.

In vivo eye irritation test

In vivo eye irritation testing was carried out using rabbits and as per Draize test protocol. Optimized formulation of bepotastine besilate ophthalmic solution 1.5% was used for this test. The formulation was found to be non-irritating with no ocular damage or abnormal clinical signs to the cornea, iris or conjunctivae observed (Table 8, 9, 10). Hence the formulation was suitable for the eye instillation.

CONCLUSION

The present study shows that laboratory prepared formulation of bepotastine besilate ophthalmic solution 1.5% has showed good stability at both 25°C and 40°C as the drug and preservative content was within the accepted range, the pH was in the range of 6.8–7 and isotonicity of the formulation is maintained throughout the stability period. Also The formulation was found to be non-irritating with no ocular damage or abnormal clinical signs to the cornea, iris or conjunctivae in the draize test protocol.

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CONFLICT OF INTEREST

Declared none

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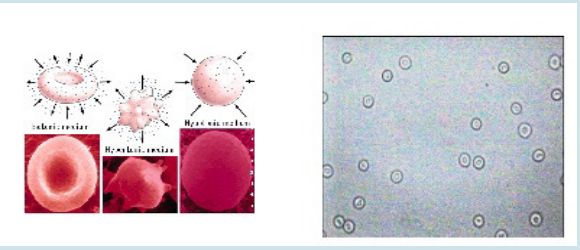
PICTORIAL ABSTRACT



SUMMARY

- Bepotatine besilate is a new second-generation antihistamine and Benzalkonium chloride (BKC), a typical quaternary ammonium salt, is often used as an antiseptic.
- Bepotastine beslitae formulation was prepared and evalued.
- Formulation optimization and evaluation is done in terms of description, pH, assay of bepotastine besilate, assay of benzalkonium chloride, viscosity, osmolarity, drop size study, water loss study, stability study which includes accelerated, long term, freeze thaw and light. Preservative efficacy study, sterility test and *in vivo* eye irritancy test are also the important parameters which are evaluated.
- The present study shows that laboratory prepared formulation of bepotastine besilate ophthalmic solution 1.5% has showed good stability at both 25°C and 40°C as the drug and preservative content was within the accepted range, the pH was in the range of 6.8-7 and isotonicity of the formulation is maintained throughout the stability period.
- The formulation was found to be non-irritating with no ocular damage or abnormal clinical signs to the cornea, iris or conjunctivae in the draize test protocol.

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