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PREPARATION, CHARACTERIZATION AND EVALUATION OF AQUEOUS EYE DROPS OF PROSTAGLANDIN ANALOGUES

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ABSTRACT

The objective of the present investigation is to Prepare, Characterize and Evaluate the aqueous eye drops of prostaglandin analogues. The eye drops were prepared by Stirring method by using over head mechanical stirrers. The different excipients were used for their compatibility with latanoprost, there was no chemical and physical interaction occurred. The Pre-formulation parameters such as API characterization, selection of excipients, selection of buffer percentage for solubilization of excipients and selection of order of addition of excipients. The description, osmolality, pH, assay, preservative content and relative substances were evaluated for eye drops. The effect of these variables on the drug release profile of latanoprost was also studied. The aqueous eye drops were formulated using 6.7 pH phosphate buffer solutions, 0.002% of Benzalkonium chloride as preservative and sodium chloride for adjusting the osmolality. Based on the evaluation results, experiment number 17 was selected as the best formulation. The results indicated that the selected formulation was stable during the test period of accelerated stability studies. All the evaluated result was found to be satisfied.

Keywords: Prostaglandin analogues, BKC, Latanoprost. Aqueous eye drops.

INTRODUCTION

Ideal ophthalmic drug delivery must be able to sustain the drug release and to remain in the vicinity of front of the eye for prolong period of time. Consequently it is imperative to optimize ophthalmic drug delivery. Ophthalmic preparations are sterile products essentially free from foreign particles, suitably compounded and packaged for instillation in to the eye. The following dosage forms have been developed to ophthalmic drugs. Some are in common use, some are merely experimental, and others are no longer used.Glaucoma is a commonly occurring disease affecting eye. There are products available in the market to treat glaucoma. Prostaglandin analogues are the recently used, very effective drugs for treating glaucoma. Latanoprost was selected for development of an eye drops.The ophthalmic

products can helpful for the treatment of glaucoma. Aqueous eye drops of prostaglandin analogues are topically administered in to the eye for use of glaucoma. They are sterile, essentially free from foreign particles. The primary mechanism by which is most prostaglandins are decreasing intraocular pressure by increasing out flow, especially through the uveoscleral out flow. Prostaglandins have been to increase out flow facility. Prostaglandin analogues are several advantages over other ocular hypotensive. Beta blockers do not reduce aqueous flow during sleep. Prostaglandin analogues potentially can reduce intraocular pressure below episcleral venous pressure, unlike medication that increases out flow facility.The active drug is solved state and may be immediately active. Prostaglandin analogues are more potent and effective ocular hypotensive agents, which once a day dosing at night. The main objective is Preparation, Characterization and Evaluation of aqueous eye drops of Latanoprost for glaucoma to reduce the intraocular pressure.

MATERIALS AND METHODS

Latanoprost procured by Matrix Laboratories Pvt Ltd.Secundrabad. Benzalkonium chloride NF, Sodium dihydrogen phosphate monohydrate USP, Disodium hydrogen phosphate anhydrous USP procured by Loba chemie,cochin,Sodium chloride USP, Water for injection procured by S.D.Fine chemicals,Mumbai. White colored HDPE caps (sterile), Clear LDPE Droppers (sterile), 5ml clear LDPE bottle (sterile) procured by Rexam France.

METHODS

MANUFACTURING ENVIRONMENT

Aside from drug safety, stability, efficacy and shelf-life consideration associated with tonicity, pH, and buffer capacity. The major design criteria of an ophthalmic solution are the additional safety criteria of sterility, preservation efficacy, and free from extraneous foreign particulate matter. These environmentally controlled must meet the requirement of class 100,000 space in all areas where open contain and closures are not exposed, or where product filling and capping operations are not taking place. Often there design criteria are coupled with laminar air flow concepts.

MANUFACTURING TECHNIQUES

Aqueous ophthalmic solutions are manufactured by methods that call for the dissolution of the active ingredient and all or portion of the excipients in to all or a portion of the water and sterilization of this solution by heat or by sterilizing filtration through sterile depth or membrane and filter receptacle. If complete at this point, such as previously sterilized solutions of viscosity-imparting agents, preservatives, and so on, and the batch is brought to final volume with additional sterile water, decreased contamination potential, lower weight, and lower cost. The plastic bottle has the dispensing tip as an integral part of the package. The plastic bottle and dispensing tip is made of low density polyethylene (LDPE) resin. The LDPE resins used are compatible with a very

wide range of drugs and formulation components. The plastic dropper bottles are also permeable to water. The disadvantage is their sorption, leaching and permeability

PACKING MATERIALS

Eye drops have been packaged almost entirely in a plastic dropper bottle. The designed plastic dropper bottle is convenience of use by the patient characteristics. This can be achieved by using a resign containing an opacifying agent such as titanium dioxide, by placing an opaque sleeve over the containers. If the drug is light sensitive, additional package protection may be required. Use of an ETO (ethylene oxide) sterilized PE, PP and/or PET container to improve the stability of an aqueous pharmaceutical composition, in particular to improve the stability of a composition being susceptible to oxidative degradation.2.5 ml filled in 5.0ml clear LDPE bottles with transparent dropper tip and capped with white HDPE cap.

EXPERIMENTAL PART

Preformulation

Preformulation testing is an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. It is the first step in the rational development of dosage forms.

Objective & Scope:

The overall objective of preformulation testing is to generate information useful to the formulation in developing stable and bioavailable dosage forms. The use of preformulation parameters maximizes the chances in formulating an acceptable, safe, efficacious and stable product.

Pre- formulation studies

Prototype Formula Selection

- BKC used for solubilization and preservation of the product.
- The BKC concentration ranges were taken as 0.006%, 0.008%, 0.01% and 0.02%. The active was completely soluble with the range of 0.02% of BKC.
- As per literature the product is stable at a pH of 6.7 and to achieve this various combinations of phosphate buffers were evaluated and found that the desired pH was achieved with the below mentioned combination
- Finally quantity of Sodium chloride was so fixed that the final formulation would achieve an osmolality of about 267mOsm/kg which is required for an ophthalmic solution.

Table No. 1. Prototype formula

S.NO	INGREDIENT	CATEGORY	QTY (mg/ml)
1	Sodium chloride	Tonicity agent	4.1
2	Sodium dihydrogen phosphate monohydrate	Buffering agent	4.6
3	Disodium hydrogen phosphate	Buffering agent	4.74
4	Benzalkonium chloride	preservative	0.2
5	Purified water	vehicle	qs-1ml

Selection of buffer volume for solubilization of API:

Different trials were taken using of quantity of buffer (% buffer of batch volume) to dissolve the API.

Selection of order of addition of excipients:

Different trails were taken with order of addition of excipients.

Table No. 2. Selection of buffer volume.

QTY. Of buffer (%) of batch size	Time required for solubilization (hours)
40%	3 hours
60%	4 hours
80%	5 hours

Table No. 3. Order of addition of excipients.

Step 1	Step2	Step3	Step 4	Observation
B.K.C.	NaCl	$NaH_2PO_4.H_2O$	$Na_2HPO_4H_2O$	Clear solution
$NaH_2PO_4.H_2O$	$Na_2HPO_4H_2O$	NaCl	B.K.C.	Clear solution
$NaH_2PO_4.H_2O$	$Na_2HPO_4H_2O$	B.K.C.	NaCl	Clear solution
NaCl**	$NaH_2PO_4.H_2O$***	$Na_2HPO_4H_2O$****	B.K.C.*	Clear solution

* B.K.C = Benzalkonium chloride ** NaCl = Sodium chloride

*** $NaH_2PO_4.H_2O$ = Sodium dihydrogen phosphate monohydrate

**** $Na_2HPO_4H_2O$ = Disodium hydrogen phosphate

CONCLUSION: Finally order of addition of excipients was so designed that initially excipients which has no impact on pH and solubilization of the active was added (Sodium Chloride). Then excipients which impact pH was added (Phosphate buffers) and lastly those impact solubilization of active (Benzalkonium chloride) was added.

FORMULATION DEVELOPMENT

TRAIL BATCH WITH API

Table No. 4. Weighing record for trail batch with API.

S. No	Ingredients	mg/ml	mg/1000ml
1	Sodium chloride	4.1	4100
2	Sodium dihydrogen phosphate monohydrate	4.6	4600
3	Disodium hydrogen phosphate	4.74	4740
4	Benzalkonium chloride	0.2	216
5	Latanoprost	0.05	50.96
6	Purified water	Q.S. to 1ml	Q.S. to1000ml

MANUFACTURING PROCESS:

I. 400ml of purified water was taken in SS vessel and dissolved weighed quantity of sodium chloride. And added sodium dihydrogen phosphate monohydrate, Disodium hydrogen phosphate to it one by one under stirring.
II. Weighed quantity of B.K.C and transferred in to SS vessel and stirred for 1hour.
III. Weighed quantity of drug and transferred in the above solution and stirred for 3hours at 350 rpm using over head mechanical stirrer.
IV. Makeup volume to 900ml and stirred it for another 1 hour.
V. Finally makeup the volume to 1litre and stirred for 1 hour.

RESULTS AND DISCUSSION

Formulation development:

Based on the pre- formulation studies on selection of qualitative and quantitative excipients, quantity of water percentage for solubilisation of excipients, order of addition of excipients and initial trail with latanoprost. The results are as follows

Table. No.12. Results of formulation development

Description	pH	Assay of API (%)
Hazy solution	6.75	94.6

There was formation of hazy solution and low assay value. This could be due to incomplete dissolution of API. Hence, next trials were taken to optimize stirring speed & time to form clear solution and achieve desire assay.

Optimization of stirring speed:

Three trails were taken at stirring rate of 500, 750, 1000 rpm. Selected stirring time was same. The results are as follows

Table. No.13. Results of Optimization of stirring speed.

Stirring speed	Description	Assay of API (%)
550 rpm	Hazy solution	95.3
750 rpm	Less hazy solution	96.6
900 rpm	Clear solution	99.4

From above data, it has been shown that the solution was clear and the assay value is 99.4% at 900 rpm. And this API gets dissolved completely at stirring speed of 900 rpm. Hence, next trials were taken to at 900rpm.

Optimization of stirring time:

After addition of excipients and API, the bulk was stirred at 1000 rpm using over head mechanical stirrer for 3hours, in-between 2ml of samples were given at different time intervals for analysis. The results are as follows

Table. No.14. Results of Optimization of stirring time

Assay (%)	1hour	2hours	3hours
API	91.6	97.0	100.7

At 3 hours desired assay was achieved and hence further trials were conducted at 900rpm and 3 hours stirring.

Optimization of temperature:

To reduce the processing time trail was taken at higher temperature at 900 rpm stirring speed and 3 hours stirring time. The results are as follows

Table. No.15. Results of Optimization of temperature

Temperature (°C)	Assay of API (%)
25°C	98.4
40°C	85.3
60°C	63.3

There was a significant drop in the assay of API. This may be due to the degradation of API at higher temperatures. Hence further trials were processed at room temperature (25°C).

Selection of sterilization process:

Since the active latanoprost is sensitive to heat (as determined above recommended storage is 2-8°C) terminal sterilization by autoclaving is not suitable for this product. Hence sterile filtration (0.22µ) was selected as method of sterilization process was evaluated and further compatibility of the product with different types of filters. The results are as follows

Table. No. 16. Results of sterilization process

ASSAY (%)	Initial	NYLON	PVDF
API	97.8	10.2	72.5

Drop in assay was predominant in Nylon filter. Hence PVDF filter was selected for further trials.

Optimization of pH:

Two trails were taken to evaluate the impact of pH at higher (7.0) and lower pH (6.0) by using 1N NaOH/1N Hcl. The results are as follows

Table. No.17. Results of Optimization of Ph

Condition	Description	pH	Assay of API (%)	BKC Content (%)	Related substances (%)	
					Single max.	Total
Initial	Clear solution	6.1	97.4	99.1	0.34	0.5
25°C/60%RH-4W	Clear solution	6.2	94.7	98.1	0.64	0.72

Condition	Description	pH	Assay of API (%)	BKC Content (%)	Related substances (%)	
					Single max.	Total
Initial	Clear solution	7.0	97.9	98.4	0.13	0.19
25°C/60%RH-4W	Clear solution	7.2	94.9	97.9	0.65	0.99

The product is stable over the pH range of 6.0 to 7.0.

Selection of packing materials:

The designed plastic dropper bottle is convenience of use by the patient, decreased contamination potential, lower weight, and lower cost.

The plastic bottle has the dispensing tip as an integral part of the package.

The plastic bottle and dispensing tip is made of low density polyethylene (LDPE) resin. The LDPE resins used are compatible with a very wide range of drugs and formulation components.

The following packing materials have been used for storage of the product.

1. LDPE bottle (Transluent-5ml)
2. LDPE with dropper tip.
3. Cap – HDPE.

Particle size & Zeta potential:

Particle size and zeta potential were measured for the final formulation (Exp. No.17).

Table. No.18. Results of Particle size & Zeta potential

Average Particle Size	509.8 nm
Average Zeta Potential	23.1 mV

The average droplet size of the formulation is in the range of nanometer and Zeta potential is below 30mV which provides desired stability characteristics to the formulation system.

Stability studies:

Using prototype formula and manufacturing process, one batch was manufactured for stability study .

FOR LDPE VAILS:

Table. No.19. Stability data.

Condition	Description	Assay (%)	BKC content (%)	Single Max unknown impurity (%)	Total impurity (%)
Initial	Clear solution	96.8	97.7	0.10	0.10
2°-8°C -4W	Clear solution	98.2	99.7	0.02	0.08
2°-8°C -12W	Clear solution	96.9	97.9	ND	1.40
25°C/60%RH-4W	Clear solution	96.1	99.4	0.02	0.08
25°C/60%RH-12W	Clear solution	96.4	97.7	ND	1.62

From the stability data, it is clear that the product is stable in accelerated conditions.

SUMMARY AND CONCLUSION

Based on the pre- formulation studies, selection of qualitative and quantitative excipients, quantity of water percentage for solubilisation of excipients, order of addition of excipients and initial trail with latanoprost. Hazy solution was formed with low assay value (94.6%). This may be due to incomplete dissolution of latanoprost.Three trails , were taken at 500, 750, 900 rpm. It has been shown that the solution was clear and the assay value is 99.4% at 900 rpm. The latanoprost dissolved completely at 900 rpm.In, after addition of excipients and latanoprost, the bulk was stirred at 900 rpm using over head mechanical stirrer for 3hours, in-between 2ml of samples were given at different time intervals for analysis. Desired assay (100.7%) was achieved at 3 hours.To reduce the processing time, trail was taken at higher temperature at 900 rpm stirring speed and 3 hours stirring time. There was a significant drop in the assay of API. This may be due to the degradation of latanoprost at higher temperatures, hence the active latanoprost is sensitive to heat terminal sterilization, and autoclaving is not suitable for this product. Sterile filtration (0.22µ) was selected for as method of sterilization process was evaluated and further compatibility of the product with different types of filters. Drop in assay (10.2%) was predominant in Nylon filter. PVDF filter was selected to achieve higher percentage assay (72.5%).Two trails were taken to evaluate the impact of pH at higher (7.0) and lower pH (6.0) by using 1N NaOH/1N Hcl. The product was stable over the pH range of 6.0 to 7.0.Particle size and zeta potential were measured for the final formulation .The average droplet size of the formulation is in the range of 509.8 nanometre and Zeta potential is below 30mV which provides desired stability characteristics to the formulation system.

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