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DEVELOPMENT OF STABLE FORMULATION OF TRAVOPROST OPHTHALMIC SOLUTION 0.004%

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ABSTRACT

Travoprost, an insoluble prostaglandin is solubilized using a combination of surfactants, Polysorbate 80 and Solutol HS 15 (Macrogol 15 Hydroxystearate) to form a micellar solution. The marketed formulation, *Travatan Z*, uses Zinc as the preservative in a patented preservative system *SofZia*, which has potential to form precipitates due to interaction of Zn with one of the surfactants. In this work, potassium sorbate is used as a preservative and acceptable anti-microbial efficacy test was demonstrated at 80% and 100% of preservative concentration. The concentrations of sodium chloride and propylene glycol were optimized using a statistical experimental run using Design of Experiments in a *Design Expert 9* software using a Central Composite Design using Response Surface method (RSM). Particle size of micelles (Z-average) and osmolality of the formulation were measured as responses. Effect of tubings, filters, sterilization of container closure system were evaluated and pH-Stability profile was generated. Bottles sterilized by ethylene oxide showed better stability than gamma sterilized bottles. The stability of the formulation is better as pH increases from 5 to 6. At an optimum pH of 5.7 the stability profile was compared with the marketed formulation *Travatan Z* and showed superior stability.

KEYWORDS: Travoprost micelles, potassium sorbate, pH-stability, preservative efficacy, LDPE bottles.

1. INTRODUCTION

Travoprost is a prostaglandin analogue widely used for reducing intraocular pressure (IOP) in patients affected with glaucoma and ocular hypertension. It exerts its ocular hypotensive

effect through the prostaglandin FP receptors, located in the ciliary muscle and the trabecular meshwork.[1] Alcon Laboratories Inc. markets this travoprost ophthalmic solution 0.004% in several countries across the globe. However, Alcon's formulation is not the same in all countries. In USA and Canada, the formulation contains the patented *sofZia* preservative system and is marketed under the brand name *Travatan Z*®. In Europe and Australia, the preservative is polyquaternium-1 and in South Africa, the preservative is benzalkonium chloride. Alcon has disclosed in patent[2] that the zinc present in the *sofZia* preservative system interacts with 12-hydroxy stearic acid and tends to form precipitate on storage. Thus the shelf life granted to Alcon for this formulation during initial review was only 14 months.[3] Alcon has also used a patented *Syndiotactic* Polypropylene bottles to minimize adsorption of drug in low density polyethylene (LDPE) bottles.[4] Detrimental effects of long-term use of quaternary ammonium compounds like benzalkonium chloride on the cornea are widely reported in the literature. Benzalkonium chloride has been reported to cause punctate keratopathy and/or toxic ulcerative keratopathy, may cause eye irritation and is known to discolour soft contact lenses.[5]

The primary objective of this work is to develop stable formulations of travoprost ophthalmic solution using regular Low density Polyethylene (LDPE) bottles without using a quaternary ammonium preservative.

2. MATERIALS AND METHODS

2.1 Materials

Travoprost was sourced from Cayman Pharma. Potassium sorbate and sodium chloride were procured from Merck. Polysorbate 80 was sourced from Croda International. Solutol HS 15 (Macrogol 15 Hydroxystearate) was a generous gift from BASF.

2.2 Chemical structure of Travoprost

IUPAC Chemical Name

Propan-2-yl (Z)-7-[(1R,2R,3R,5S)-3,5-dihydroxy-2-[(1E,3R)-3-hydroxy-4-[3-trifluoromethyl)phenoxy]-1-buten-1-yl]cyclopentyl]-hept-5-enoate

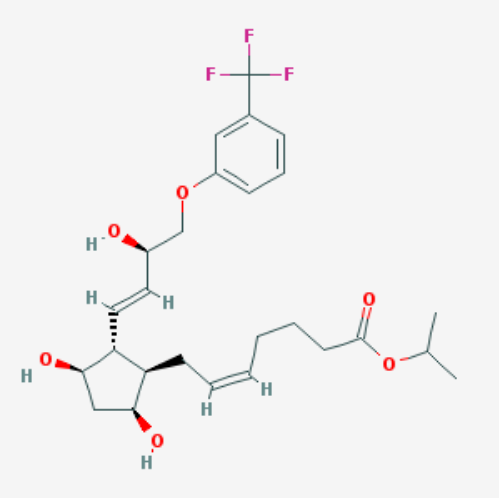

Fig. 1: Structure of Travoprost.

2.3 Potential impurities in Travoprost drug substance

The potential impurities and degradation products[6] that are controlled in the drug substance are listed below.

1. Travoprost related compound A (NMT 0.2%)- Arising from synthesis and degradation (hydrolytic)
2. Epoxy derivative (NMT 0.4%) - Arising from synthesis
3. 15-epi diasteriomer(NMT 0.1%) - Arising from synthesis
4. 5,6-trans isomer (NMT 3.5%) - Arising from synthesis and degradation
5. 15-keto derivative (NMT 0.3%) - Arising from synthesis and degradation (oxidation)
6. Any other individual impurity (NMT 0.1%)
7. Total impurities (NMT 4.0%)

2.4 Drug product specifications

The potential degradation impurities that are listed in the drug substance specifications are monitored in the drug product throughout its shelf life. The specifications of related substances in the drug product, travoprost ophthalmic solution 0.004% and its justifications are listed in below. The known impurities are controlled at limits specified in the USP monograph[7], and the unidentified impurities are controlled at ICH qualification threshold limit based on daily dose of product.

1. Travoprost related compound A – NMT 1.0% (USP limits)
2. 5,6-trans isomer - NMT 5.0% (USP limits)
3. 15-keto derivative - NMT 1.0% (USP limits)
4. Any other individual impurity - NMT 1.0% (ICH qualification threshold)

5. Total impurities (excluding Impurity A) - NMT 5.5% (USP limits)

2.5 Formulation development of travoprost ophthalmic solution 0.004%

In order to develop an alternate formulation, experimental runs using *Design expert 9* software were run to select a surfactant combination that forms stable micellar solutions. The authors have published this work earlier[8] and a combination of 0.1% each of Solutol HS 15 and polysorbate 80 were chosen for further formulation development. Propylene glycol acts as a co-solubilizer and helps to keep travoprost in solubilized state and also acts as a tonicity building agent. Potassium sorbate was chosen as the preservative as it is one of the safer preservatives as compared the commonly used quaternary ammonium compounds like benzalkonium chloride and it is most effective below pH 6.0. A concentration of 0.47% was used as it is approved for ophthalmic solutions at this level, in the 'Inactive ingredient database' of USFDA. Sodium chloride was chosen as the tonicity builder, in addition to propylene glycol. The concentrations of sodium chloride and propylene glycol were varied using a statistical experimental run using Design of Experiments in a *Design Expert 9* software. A Central Composite Design (CCD) using Response Surface method (RSM)[9] was used to optimize concentrations of sodium chloride and propylene glycol. Since propylene glycol acts as a co-solubilizer, it could affect the particle size of micelles as well as osmolality. Sodium chloride would only affect the osmolality of the finished product. Hence particle size and osmolality were chosen as the responses. Ten experiments including two center points with various concentrations of sodium chloride and propylene glycol were performed. (Table 1). The concentrations of other ingredients in the formulation were kept constant.

Table 1: Experimental run for optimization of sodium chloride and propylene glycol concentrations with responses.

Run order	Factor 1	Factor 2	Response 1	Response 2
	sodium chloride (%)	propylene glycol (%)	particle size, nm (Z-average)	osmolality (mOsmols/Kg)
1	0.30	0.80	14.92	314
2	0.35	0.75	13.98	278
3	0.40	0.60	17.43	276
4	0.28	0.70	15.32	253
5	0.35	0.84	12.38	294
6	0.42	0.70	15.46	293
7	0.30	0.60	15.22	237
8	0.35	0.75	15.09	280
9	0.40	0.80	15.6	303
10	0.35	0.56	17.25	237

2.6 Measurement of average particle size (Z-average)

Particle size measurement was carried out using Sympatec nanophox instrument which employs the principle of Dynamic Light Scattering (DLS) to measure nano particles in the range of 1nm to 10,000 nm. Mean particle size, Z-average was measured on undiluted samples of Travoprost ophthalmic solution. The results are tabulated in table 3.

2.7 Measurement of osmolality

Osmolality was measured on undiluted samples using an Osmometer- Model 3250 of Advanced Instruments, Inc. This instrument uses the principle of measuring osmolality precisely by measuring the difference in freezing point depression due to presence of solutes in the test product and in solvent alone. The results of osmolality are tabulated in table 3.

2.8 Statistical analysis (ANOVA)

The results of particle size and osmolality were evaluated statistically using regression analysis. The following polynomial equations were generated, in which the model F ratios were statistically significant at $\alpha < 0.05$, with a statistically non-significant lack of fit at $\alpha > 0.05$. The model for statistical analysis of particle size is *Response surface linear* model and that for osmolality is *Response surface reduced cubic* model.

Z-average particle size = +20.45308+7.71997*A - 11.27153*B (1)

Osmolality = -5661.39773 + 41951.22657*A + 4183.42895*B - 50352.27279*AB-67025.25253*A2+6130.74494*B+96928.93219*A^2B-14284.27125*AB^2 (2)

Where A= Concentration of Sodium Chloride and B= Concentration of Propylene glycol.

The results of statistical analysis for Z-average particle size and Osmolality is tabulated in Table 2. The response surface plots and the Contour plots showing the effect of sodium cChloride and Propylene glycol on Z-Average particle size are shown in Figures 2A and 2B respectively and effect on osmolality are shown in figures 2C and 2D respectively.

Table 2: Results of statistical analysis (ANOVA) of the experimental design.

Responses	Model p value	Adequate precision	Lack of fit test; p value
Z-avg particle size	0.0403	5.604	0.5071
Osmolality	0.0015	83.518	0.8440

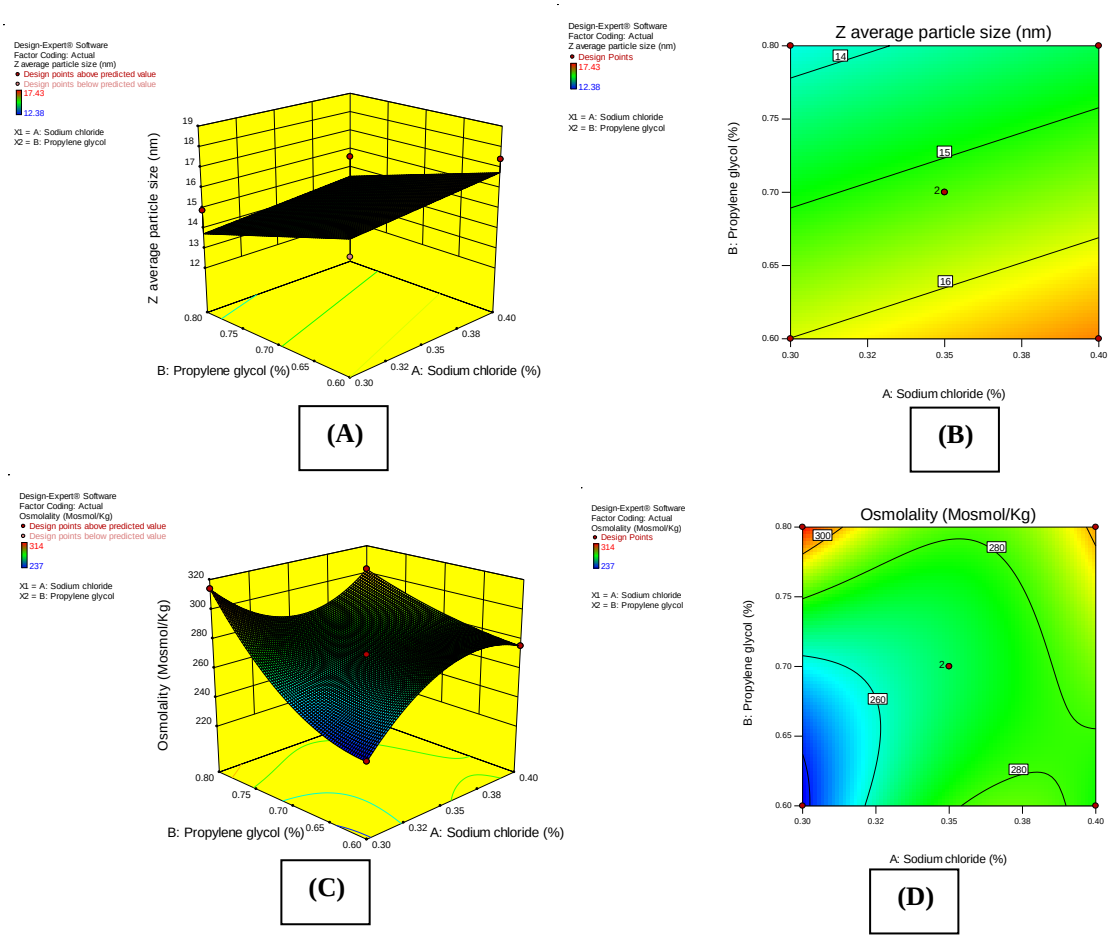

Fig 2: (A) and (B) – Response surface plot and Contour plots showing of effect of sodium chloride and propylene glycol on Z-Average particle size; (C) and (D) - Response surface plot and Contour plots showing of effect of sodium chloride and propylene glycol on Osmolality.

2.9 Final formulation of travoprost ophthalmic solution 0.004%

From the DOE results, concentration of 0.35% sodium chloride and 0.75% of propylene glycol gave an osmolality of 278-280mOsmols/Kg. Human tears have been disclosed to be isotonic to plasma.[10] Though eyes can tolerate a wide range of osmolality without marked discomfort, most ophthalmic formulations that are marketed have osmolality between 275 to 295 mosmols/Kg close to osmolality of human plasma.[11] The concentrations of sodium chloride and propylene glycol finalized are 0.35% and 0.75% respectively. The final formula is listed in Table 3.

Table 3: Formulation of travoprost ophthalmic solution 0.004%.

Sr. No.	Ingredients	mg/ml
1	Travoprost	0.04
2	Potassium sorbate	4.7
3	Sodium chloride	3.5
4	Propylene glycol	7.5
5	Polysorbate 80	1.0
6	Solutol HS 15	1.0
7	O.1N HCl	q.s
8	Purified water	q.s

q.s- quantity sufficient.

2.10 Selection of filter and tubing for filtration of travoprost ophthalmic solution

Filter and tubings that are used during the aseptic manufacturing and filtration sterilization of the finished product could have an impact on the assay of travoprost and assay of potassium sorbate due to possible adsorption on the filter, as well as on the related substances test due to possible leaching from tubings and filter. To assess the impact on tubings, the product was filled in three different tubings, namely, Platinum cured silicon tubes, Perfluoroalkoxy tubes (PFA) and Silicon tubes, sealed at both ends and kept standing for 24 hours. Samples were analyzed after 12 hours and 24 hours for assay of travoprost, assay of potassium sorbate and related substances. For assay of travoprost and related substances test, analysis was carried out using USP methods and for assay of potassium sorbate, an in house HPLC method was developed. To assess the impact of filters, three different filters, namely, 0.22µ Polyvinylidene Fluoride filter (PVDF), 0.2µ Nylon filter and 0.2µ Polyether sulfone filter (PES), were dipped in travoprost solution and analyzed for assay of travoprost, assay of potassium sorbate and related substances after 12 and 24 hours The results were compared with unfiltered bulk analysis. The results of tubing study is tabulated in table 4 and the results of filter study is tabulated in Table 5.

Table 4: Effect of tubing material on assay of travoprost, potassium sorbate and on related substances of travoprost ophthalmic solution 0.004%.

Physical Parameters	Initial unfiltered	Platinum cured Silicon tube		PFA tube		Silicon tube	
		12 hr	24 hr	12 hr	24 hr	12 hr	24 hr
Assay of travoprost (%)	99.6	90.9	91.3	98.2	96.5	86.2	81.0
Assay of potassium sorbate (%)	100.2	100.9	99.6	103.4	101.8	98.4	98.1
Related Substances							
5, 6 Trans isomer	**0.1**	0.1	0.1	0.08	0.09	ND	ND
15 - Keto Derivative	**0.12**	0.09	0.08	0.13	0.12	0.12	0.13
Related compound A	**ND**	ND	ND	ND	ND	ND	ND
Single Maximum Unknown	**0.11**	0.12	0.18	0.1	0.16	5.3	5.6
Total Impurities	**0.33**	0.39	0.48	0.4	0.66	13.0	12.2

ND- Not detected.

Table 5: Effect of filter material on assay of travoprost, potassium sorbate and on related substances of travoprost ophthalmic solution 0.004%.

Physical Parameters	Initial unfiltered	0.22 µ PVDF		0.2 µ Nylon 66		0.2 µ PES	
		12 hr	24 hr	12 hr	24 hr	12 hr	24 hr
Assay of travoprost (%)	99.6	98.2	97.8	93.5	93	97.8	97.5
Assay of potassium sorbate (%)	100.2	103.5	104.4	101.8	100.1	102.7	101
Related Substances							
5, 6 Trans isomer	**0.1**	0.09	0.09	0.08	ND	ND	0.10
15 - Keto Derivative	**0.12**	0.12	0.11	0.11	0.11	0.11	0.10
Related compound A	**ND**	ND	ND	ND	ND	ND	ND
Single Maximum Unknown	**0.11**	0.12	0.12	0.1	0.11	0.10	0.11
Total impurities	**0.33**	0.33	0.32	0.29	0.22	0.21	0.31

ND- Not detected.

2.11 Method of manufacture

Travoprost is dissolved in a stock solution of propylene glycol, Solutol HS 15 and polysorbate 80 in water. A solution of sodium chloride and potassium sorbate in water is prepared and added to the travoprost stock solution and mixed well. pH is adjusted with 0.1N HCl between 5.4 and 6.0. The solution is aseptically filtered through 0.22µ Polyethersulfone membrane (PES) and filled in low density polyethylene vials (LDPE) fitted with LDPE droppers and high density polyethylene cap (HDPE).

2.12 Sterilization of container closure system

Before starting formal stability studies on the final formulation, it is important to know the impact of sterilization technique of the container closure system (CCS) on the product stability. The container closure system selected for travoprost ophthalmic solution 0.004% is white opaque LDPE vials with transparent nozzles and HDPE caps. Sterilization of CCS using two different techniques was done i.e. ethylene oxide gaseous sterilization and gamma irradiation. The finished product was filled in both these containers and charged on stability at accelerated conditions of 40°C/25% RH. Data is tabulated in Table 6.

Table 6: Effect of Sterilization of CCS on related substances of travoprost ophthalmic solution 0.004%.

Test	Limits	Initial	40°C/25%RH	
			2M ETO	2M Gamma
Assay of travoprost	90.0-110.0%	100.5	97.7	95.7
Assay of potassium sorbate	90.0-110.0%	100.6	96.3	95.2
Related substances				
Travoprost related compound A	NMT 1.0%	0.07	0.52	0.55
5,6-trans isomer	NMT 5.0%	ND	ND	ND
15-keto derivative	NMT 1.0%	ND	0.32	0.53
Single maximum unknown	NMT 1.0%	0.3	0.57	0.6
Total impurities	NMT 5.5%	1.1	2.8	3.5

NMT – Not more than; ND – Not detected.

2.13 Effect of pH on the finished product stability

The finished product was manufactured at four different pH to study effect on pH on impurity profile of the finished product, namely pH 5.0, pH 5.4, 5.7 and 6.0. Comparative data of initial time point and after six months at accelerated conditions of 40°C/25% RH is tabulated in Table 7 and graphically represented in Fig 3.

Table 7: Effect of pH on related substances of travoprost ophthalmic solution.

Test	Limits	Initial				6 M 40°C/25%RH			
		pH 5.0	pH 5.4	pH 5.7	pH 6.0	pH 5.0	pH 5.4	pH 5.7	pH 6.0
Assay of travoprost	90.0-110.0%	99.1	96.8	100.5	100.7	106.9	95.8	98.7	104.0
Assay of potassium sorbate	80.0-110.0%	99.8	100.5	100.4	101.6	85.4	87.3	97.7	103.1
Related substances									
Travoprost related compound A	NMT 1.0%	0.08	ND	ND	ND	1.5	0.63	0.36	0.41
5,6-trans isomer	NMT 5.0%	ND	ND	ND	ND	0.15	0.1	0.16	0.17
15-keto derivative	NMT 1.0%	ND	0.09	0.10	ND	0.19	ND	ND	0.09
Single maximum unknown	NMT 1.0%	5.7	BLOQ	0.11	0.15	7.9	0.36	0.54	ND
Total impurities	NMT 5.5%	6.2	0.77	0.21	0.41	9.7	1.5	1.4	0.67

ND – Not detected; BLOQ – Below LOQ.

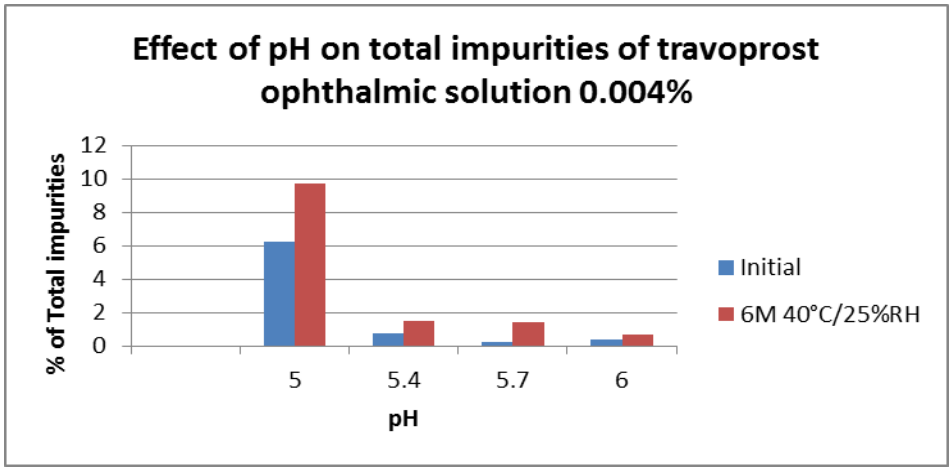

Fig. 3: Effect of pH on total impurities of travoprost ophthalmic solution 0.004% at 6 months accelerated stability.

2.14 Antimicrobial efficacy test (AET) of potassium sorbate in the finished formulation

Two batches of travoprost solution were manufactured as per formula in table 5 at 100% and 80% concentration of potassium sorbate, in order to assess its preservative effectiveness as per USP. Antimicrobial preservative testing at lower concentration of preservative i.e. 80% of label claim is tested, as there is a drop in levels of potassium sorbate at lower pH of 5.0 and 5.4 to around 85% of the label claim at the end of 6 months accelerated stability at 40ºC/25%RH.

The results of AET test for 100% and 80% preservative concentration are tabulated in Tables 8 and 9 respectively.

Table 8: Summary results of antimicrobial effectiveness test at 100% concentration of potassium sorbate.

Name of microbial culture Bacteria	**Log reduction in viable count from initial calculated viable count at '0' hour**		**Log of viable count at 28 days**	**USP compliance**
	After 7 days (Limit: NLT 1)	**After 14 days (Limit: NLT 3)**	**Limit: No increase from 14 days**	
Escherichia coli ATCC 8739	4.25	6.53	No increase	Complies
Pseudomonas aeruginosa ATCC 9027	4.27	6.48	No increase	Complies
Staphylococcus aureus ATCC 6538	4.26	6.52	No increase	Complies
Yeasts and Molds	**Log of viable count at 7 days**	**Log of viable count at 14 days**	**Log of viable count at 28 days**	**USP compliance**
	(Limit : No increase form '0'hr)	**(Limit : No increase form '0'hr)**	**(Limit : No increase form '0'hr)**	
Candida albicans ATCC 10231	No increase	No increase	No increase	Complies
Aspergillus brasiliensis ATCC 16404	No increase	No increase	No increase	Complies

Table 9: Summary results of antimicrobial effectiveness test at lower concentration of potassium sorbate (80% of label claim).

Name of microbial culture Bacteria	Log reduction in viable count from initial calculated viable count at '0' hour		Log of viable count at 28 days	USP compliance
	After 7 days (Limit: NLT 1)	After 14 days (Limit: NLT 3)	Limit: No increase from 14 days	
Escherichia coli ATCC 8739	3.98	6.53	No increase	Complies
Pseudomonas aeruginosa ATCC 9027	4.03	6.48	No increase	Complies
Staphylococcus aureus ATCC 6538	4.00	6.52	No increase	Complies
Yeasts and Molds	**Log of viable count at 7 days**	**Log of viable count at 14 days**	**Log of viable count at 28 days**	**USP compliance**
	Limit : No increase form '0'hr	**Limit : No increase form '0'hr**	**Limit : No increase form '0'hr**	
Candida albicans ATCC 10231	No increase	No increase	No increase	Complies
Aspergillus brasiliensis ATCC 16404	No increase	No increase	No increase	Complies

2.15 Comparative stability studies with brand product, *Travatan Z*

The test formulation given in Table 5 was filled in 5 ml ETO sterilized Low density Polyethylene (LDPE) bottles fitted with LDPE nozzles and turquoise colored polypropylene caps and charged on accelerated stability conditions of 40ºC/25%RH and at 25ºC/40%RH and 2-8ºC as per ICH guidelines[12] for semi-permeable containers. The brand product, *Travatan Z* was charged only at accelerated conditions. The results are summarized in Table 10.

Table 10: Comparison of stability results of marketed formulation with the test product.

Tests	Limits	*Travatan Z®			Test product				
		Initial	40°C/25%RH		Initial	40°C/25%RH		25°C/40%RH	2-8ºC
			3 M	6 M		3M	6M	6M	6M
% Assay of travoprost	90.0 -110.0	101.1	100.9	104.9	100.5	100.1	98.7	102.5	98.7
pH	5.4-6.0	5.67	NP	5.83	5.7	5.9	6.0	5.84	5.78
Osmolality (mOsmols/Kg)	260-300	278	NP	283	278	284	284	282	287
Related substances									
travoprost related compound A	NMT 1.0%	ND	0.06	0.15	ND	0.16	0.36	0.2	0.04
5,6-trans isomer	NMT 5.0%	3.2	3.2	2.9	ND	0.16	0.16	ND	ND
15-keto derivative	NMT 1.0%	0.11	0.14	0.17	0.10	ND	ND	ND	ND
Single maximum unknown	NMT 1.0%	0.97	3.6	6.4	0.11	0.31	0.54	0.34	0.3
Total impurities	NMT 5.5%	4.3	7.6	11.3	0.21	1.0	1.4	1.1	1.2

* Marketed in USA by Alcon Laboratories Inc.; ND- Not detected; NP-Not performed.

3. RESULTS AND DISCUSSION

3.1 Evaluation of results of DOE

Model p value in Table 4 indicates that there is only a 4.03% chance that the model F value of the model equation (1) for Z average particle size, occurred due to noise. For Osmolality, the model p value indicates that there is only a 0.15% chance that the model F value of the model equation (2) occurred due to noise. Adequate precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. For both responses, ratios were higher than 4, indicating adequate signal. The p values of the lack of fit test for both models were not significant, indicating that these model equations fitted the data well and can be used for prediction.

The results of Z average particle size for the experimental runs ranged between 12.38 to 17.43 nm. The positive sign in equation 1 for sodium chloride and negative sign for propylene glycol indicates that the concentration of Sodium Chloride is directly proportional and that of propylene glycol is inversely proportional to particle size. This is expected as propylene glycol helps in solubilizing travoprost and thus lowering the particle size of the micelles at higher concentrations. For osmolality, the results were in the range of 237 to 314 mOsmols/Kg. Concentration of 0.35% sodium Chloride and 0.75% of propylene glycol gave an osmolality of 278-280mOsmols/Kg and was found to be optimum for an ophthalmic solution.

3.2 Filter and tubing selection

3.2.1 Tubing study

The results in Table 6 show around 8-9% of adsorption of travoprost in platinum cured tubing after 12 and 24 hours of tubing contact study. In PFA tubing after 12 hours, the adsorption is negligible. However, after 24 hours, around 3% of travoprost gets adsorbed onto the PFA tubing. With silicon tubings, maximum adsorption of travoprost was observed, around 13% and 18% in 12 hours and 24 hours respectively. The rise in related substances also is significantly high in silicon tubings compared to the other two tubings.

In all the tubings, adsorption of potassium sorbate is negligible. There is a slight increase in total impurities after 12 and 24 hours of exposure in platinum cured silicon tubes and in PFA tubes. However, in silicon tubings, there is significant increase in unknown impurity as well as in the level of total impurities. Based on the results of the tubing study, PFA tubings were finalized for further development as it showed minimum adsorption at 12 hours time point.

This study reflects a worst case scenario for filtration as the solution is filled in the tubes and kept for 12 hours and 24 hours. In actual production runs, during filtration, the solution will be constantly in motion and hence contact time of solution in the tubings will be minimal. However, based on the results, the filtration time will be restricted to not more than 12 hours during manufacturing.

3.2.2 Filter selection

There is no significant change in assay of travoprost and potassium sorbate in PVDF and PES filters. However, in Nylon 66 filter, significant adsorption of around 6.6% of travoprost is observed No significant increase in impurities were observed in any of the filter studied. PES was finalized for further studies over PVDF due to ease of availability and available inventory.

3.3 Sterilization of container closure system

Results of 2 month samples showed that product filled in gamma irradiated CCS showed significantly higher percentage of 15-keto derivative than those filled in ETO sterilized ones. This impurity is formed due to oxidation. The higher percentage of 15-keto impurity in gamma sterilized CCS, could be due to residual free radicals generated during sterilization of CCS triggering an oxidative reaction. Based on the data, ETO sterilized CCS were chosen for conducting stability studies as per ICH guidelines.

3.4 Effect of pH on stability of travoprost ophthalmic solution

The results of related substances at different pH of the formulation from pH 5.0 to 6.0 shows significant increase in the values of total impurities with decrease in pH. At pH 5.0, initial results for total impurities is higher than the limit of 5.5% due to significant increase in unknown impurity. However, there is no drop in assay of travoprost and mass balance is not achieved. This could be due to different chromophores present in the unknown impurity giving very different response as compared to travoprost in the chromatographic method. At pH 5.7 and pH 6.0, the results of related substances is satisfactory and well within the acceptance criteria. Potassium sorbate is known to be most effective below pH 6.0. Hence the pH range selected for travoprost ophthalmic solution is 5.4 to 6.0, with an optimum pH of 5.7.

3.5 Antimicrobial Efficacy test results

Test results of both formulations, one containing 100% of potassium sorbate and second one containing 80% of potassium sorbate levels in the formulation were within the USP acceptance criteria for all the specifies bacteria and yeasts and fungi. Thus potassium sorbate in the formulation acts effectively as a preservative.

3.6 Comparison of stability data with brand product

There is no significant difference in assay of travoprost in the test product across all stability conditions as well as in the brand product, *Travatan Z.* Travoprost related compound A is the free acid degradant of travoprost. It is formed by the hydrolytic cleavage of the isopropyl ester group from the travoprost molecule. In the brand product the free acid degradant increases from below detection level to 0.15% at 6 months accelerated conditions. However, in the test product, though the free acid impurity is slightly higher than the brand product and increases from below detection limit to 0.36% at 6 months accelerated conditions, it is still well within the USP acceptance criteria of not more than 1.0%. The 5, 6-trans isomer is a degradation product formed by thermal conditions. The values of this impurity for test product is better than the brand product, though both are within USP limits of 5.0%. 15-keto impurity is an oxidative impurity and controlling this impurity at low levels require extensive controls on the oxygen content of the solution during manufacturing as well as nitrogen purging in the bottles during filling operations. The brand product has used a specific resin, syndiotactic polypropylene to contain adsorption of API and to control oxidation of travoprost. The brand product was assigned a shelf life of only 14 months in the initial approval and requires storage at 2-25ºC. Surprisingly, the test product that was developed, did not require any controls of nitrogen flushing during manufacturing or filling and showed no detectable levels of 15-keto impurity even at accelerated conditions up to 6 months at optimum pH of 5.7.

4. CONCLUSION

A stable formulation of travoprost was developed using principles of quality by design. A simple manufacturing process, without the need for nitrogen flushing in manufacturing or the need to control oxidation by any means, was developed. Factors affecting the critical quality attributes of the product were studied to mitigate risks. The product is stable up to 6 months at accelerated conditions in low density polyethylene bottles. Thus, a proposed shelf life of 24

months can be assigned to the product without the need for any special storage conditions, which translates to significant commercial benefits with respect to logistics as well as costs.

Disclosures

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