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Effect of Formulation Factors on *in vitro* TRANSCORNEAL Permeation of Olopatadine hydrochloride aqueous Eye drops

Rajkishore Mohanty[1], Ajit Kumar Acharya[1]*, Gitanjali Mishra[2]

[1]Royal College of Pharmacy and Health Sciences, Berhampur-760 002, Orissa, India.
[2]P.G. Department of Zoology, Berhampur University, Berhampur-760007, Orissa, India.

*Corres. Author: ajit417@gmail.com ,ajit417@rediffmail.com
Mobile No: +919777932324, +919439721819, +919040303756

Abstract: The purpose of this research was to optimize the formulation factors for maximum in vitro permeation of olopatadine hydrochloride from aqueous drops through excised goat cornea and to evaluate the permeation characteristics of drug from selected marketed eye drop formulations. Aqueous isotonic ophthalmic solutions of olopatadine hydrochloride of different concentrations like 0.1%, 0.2%, and 0.3% (w/v) of (pH 7) or 0.1% (w/v) solutions of different pH or 0.1% solutions (pH 7) containing different preservatives were made. Permeation studies were conducted by taking 1 ml of formulation on the cornea (0.67 cm^2) fixed between the donor and receptor compartments of an all-glass modified franz diffusion cell and measuring olopatadine concentration in the receptor (containing 10 ml bicarbonate ringer at 37°C under stirring) by spectrophotometry at 299 nm, after 120 minutes. Increasing drug concentration in the formulation resulted in an increase in the quantity permeated but a decrease in percentage permeation. Raising the pH of the formulation from pH 6 to pH 7 increased drug permeation and the in vitro ocular availability but after that it decreases upto pH 8, indicating pH dependent transport. Compared with control formulation, olopatadine hydrochloride 0.1% (w/v) solution (pH 7) containing benzalkonium chloride (BAK; 0.01% W/V) produced higher permeation, similarly formulation containing benzyl alcohol (BA 0.5% W/V) also showed higher permeation. But formulation ontaining combination of preservatives (BAK; 0.01% W/V and EDTA 0.01% W/V) showed highest permeation. The results suggest that olopatadine hydrochloride 0.1% ophthalmic solution (pH 7) containing Benzalkonium chloride (BAK; 0.01%W/V) and EDTA (0.01% W/V) provides highest *in vitro* ocular availability through goat corneas among all the formulations including marketed products like olopine, olopat.
Keywords: Olopatadine, concentration, pH, preservative, cornea, permeation.

INTRODUCTION:

The topical therapy with antihistamines has been proven for ocular disorders like allergic conjunctivitis, keratitis, inflammation and redness of eye [1]. Antihistamines act by blocking the H_1 receptor [2-3]. Rapid and efficient drainage by the nasolachrymal apparatus, noncorneal absorption, and relative impermeability of the cornea to both hydrophilic and hydrophobic molecules, all account for poor ocular bioavailability [4-6]. The use of sustained drug delivery systems with maximizing corneal drug absorption and minimizing precorneal drug loss can solve the problem of poor bioavailability [7-10]. Research was carried out to optimize the formulation factors (e.g., concentration, pH, presence of

preservatives) for maximum in vitro permeation of olopatadine hydrochloride from aqueous drops through excised goat cornea and to evaluate the permeation characteristics of drug from selected marketed eye drop formulations.

MATERIALS AND METHODS:

Olopatadine hydrochloride was obtained from Ranbaxy laboratories (Gurgaon, India) as gift sample. Benzalkonium chloride, phenyl mercuric nitrate, Thiomersal, Nitromersal, Cetrimide, Methyl paraben, Propyl paraben were obtained from Loba Chemie Pvt. Ltd, Mumbai, The rest of the materials (analytical grade) were obtained from central drug house (New Delhi, India). Marketed Olopatadine hydrochloride eye drops- Olopat (Ajanta Pharmaceuticals Ltd, Mumbai, India), Olopine (Intas Pharmaceuticals Ltd, India) were procured from a local market. Fresh eyeballs of goat were obtained from a local butcher shop within 1 hour of the animals slaughtering.

PREPARATION OF TEST SOLUTIONS:

The required amount of Olopatadine hydrochloride was dissolved in a sufficient amount of distilled water, sodium chloride was added to make the final solution isotonic .The pH of the solution was adjusted by using 0.1 N NaOH and 0.1 N HCl, and distilled water was added to bring the final volume up to 100 ml . Creating solutions of 0.1%, 0.2%, and 0.3% (w/v) concentration at pH 7.0, similarly by keeping the concentration constant (which showed best permeation i.e., 0.1% w/v), the pH has been varied to find out the effect of pH. After optimizing the concentration and pH of the formulation (i.e., 0.1%w/v, and pH 7.0), the formulations were prepared with different preservatives by the method mentioned as above.

PERMEATION STUDY:

Freshly excised goat cornea (available for diffusion was 0.67 cm^2) was used in an all-glass modified Franz diffusion cell with the receptor compartment containing 10 mL of freshly prepared bicarbonate ringer solution, and donor with formulation (1mL). Samples were withdrawn from the receptor and analyzed for drug content by UV-spectrophotometer at 299 nm.

At the end of the experiment, each cornea was weighed, soaked in 1 ml of methanol, dried overnight at 90°C and reweighed. From the difference of weights corneal hydration was calculated

SURFACE TENSION MEASUREMENT:

To explore any possible relationship between surface tension of formulation and corneal penetration, surface tension of all the formulations were measured by Stalagmometer.

IN VITRO TITRATION:

One mL of all the formulations were titrated with 0.1N NaOH to a phenolphthalein end point and colour change from colourless to pink was taken into account.

RESULTS AND DISCUSSION:

An increase in drug concentration in the drops resulted in an increase in amount permeated after 120 minutes (i.e. 0.1466 mg for 0.1%w/v to 0.2333 mg for 0.3%w/v), but the percentage of permeation or in vitro ocular availability decreased from 14.66% to 7.77 respectively. Only the amount of drug required to saturate the epithelium would be able to partition through the stroma and endothelium to the receptor. An increase in the pH of the ophthalmic solution from pH 6 to pH 7 increased both the amount of drug permeated and the in vitro ocular availability, indicating a pH dependent transport. Olopatadine is a basic drug, will be in unionised form as the pH of the formulation is shifted towards the neutrality resulting in increased permeation. Transport of Olopatadine hydrochloride, across excised goat cornea has also been found to be pH-dependent, with a maximum at pH 7.0.

Formulation with Benzalkonium chloride (BAK; 0.01% w/v) showed significantly ($P < 0.05$) higher permeation (i.e. 18 %) than did the control formulation (i.e. 14.5%) without preservatives. Combination of Benzalkonium chloride and EDTA each having 0.01% w/v, increased the amount permeated (0.385mg) and the % permeation (38.5%) of Olopatadine the most. The optimized formulation showed the highest permeation, followed by olopine, olopat. The surface tension of the marketed formulation varied between 22.78 and 36.30 dynes/cm, indicating the presence of a surfactant. All the formulations the corneal hydration between 75% to 80%, indicating no corneal damage. The titration study confirmed the absence of a buffer in marketed preperations like olopine, olopat and optimized formulation as consumed 0.08ml of NaOH.

Table 1 – Effect of Concentration on permeation of Olopatadine hydrochloride through excised Goat Cornea

Sl. No.	Conc. of drug (%w/v)	Amount permeated (mg)	% Permeation	Corneal Hydration (%)
1	**0.1**	**0.1466 ± 0.00**	**14.66**	**78.78 ± 0.21**
2	**0.2**	**0.1916 ± 0.001**	**9.58**	**77.77 ± 0.48**
3	**0.3**	**0.2333 ± 0.002**	**7.77**	**76.89 ± 0.65**

*Values are mean ± SE of 3 corneas in each group

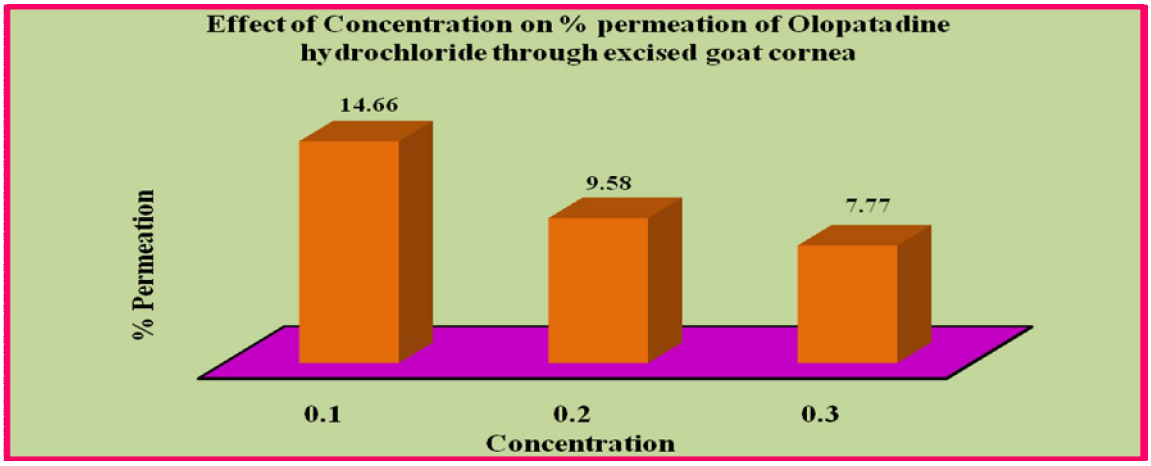

Figure 1 - Effect of Concentration on % permeation of Olopatadine hydrochloride

Table 2 – Effect of pH of Olopatadine hydrochloride aqueous solution (0.1%w/v) on permeation of drug through Excised Goat Cornea

Sl. No.	Conc. of drug (%w/v)	pH	Amount Permeated (mg)	% Permeation	Corneal Hydration (%)
1	0.1	6.0	0.081 ± 0.0085	8.16	76.52 ± 0.297
2	0.1	6.5	0.078 ± 0.0070	7.83	79.29 ± 0.345
3	0.1	6.8	0.121 ± 0.0015	12.16	78.57 ± 0.500
4	0.1	7.0	0.145. ± 0 .0013	14.5	78.37 ± 0.200
5	0.1	7.2	0.139 ± 0.009	13.91	79.73 ± 0.730
6	0.1	7.4	0.101 ± 0.0285	10.16	75.72 ± 0.237
7	0.1	8.0	0.137 ± 0.001	13.71	75.10 ± 0.105

* Values are mean ± SE of 3 corneas in each group

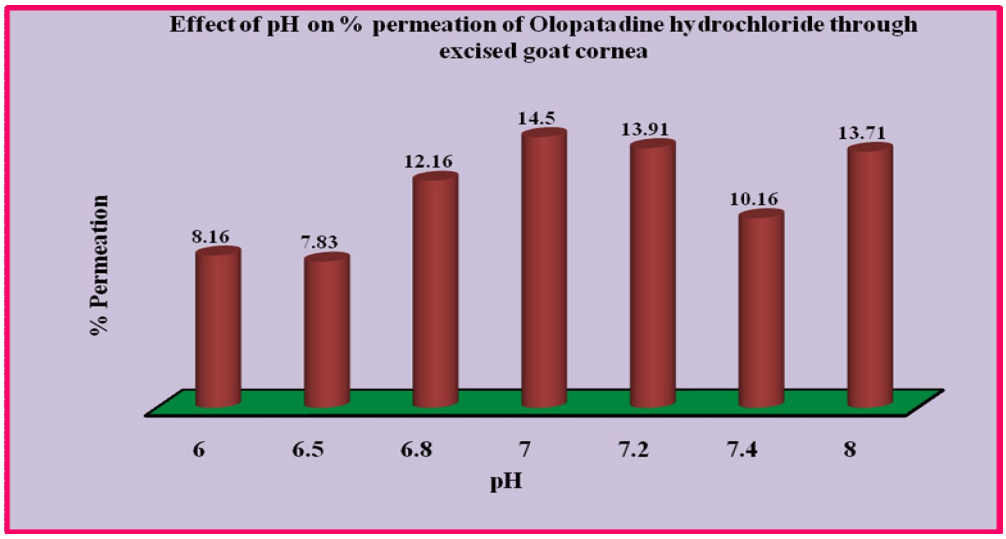

Figure 2 - Effect of pH on % permeation of Olopatadine hydrochloride

Table 3 – Effect of Preservative on permeation of Olopatadine hydrochloride from 0.1% aqueous solution (pH 7.0) through Excised Goat Cornea

Sl. No.	Conc. of drug (%w/v)	Preservative Conc. (%w/v)	Amount Permeated (mg)	% permeation	Corneal Hydration (%)
1	0.1	None	0.146 ± 0.00	14.66	78.37 ± 0.2
2	0.1	Benzalkonium chloride(BAK, 0.01)	0.180 ± 0.001	18.0	81.42 ± 0.65
3	0.1	EDTA (0.01)	0.130 ± 0.002	13.0	80.65 ± 0.65
4	0.1	Benzyl alcohol (BA,0.5)	0.186 ± 0.003	18.66	79.56 ± 0.81
5	0.1	Cetrimide (0.01)	0.121 ± 0.003	12.16	76.65 ± 0.65
6	0.1	Thiomersal (THM,0.005)	0.096 ± 0.002	9.66	81.2 ± 0.95
7	0.1	Nitromersal (NIM,0.005)	0.102 ± 0.001	10.23	78.75 ± 0.72
8	0.1	Phenyl mercuric acetate (PMA,0.002)	0.168 ± 0.002	13.25	80.07 ± 0.2
9	0.1	Phenyl mercuric nitrate (PMN,0.002)	0.091 ± 0.002	9.16	83.45 ± 0.17
10	0.1	Methyl paraben + Propyl paraben (0.02 + 0.01)	0.120 ± 0.003	12.0	80.0 ± 0.06
11	0.1	BAK+EDTA (0.01 + 0.01)	0.385 ± 0.001	38.5	77.85 ±.0.81

***** Values are mean ± SE of 3 corneas in each group.

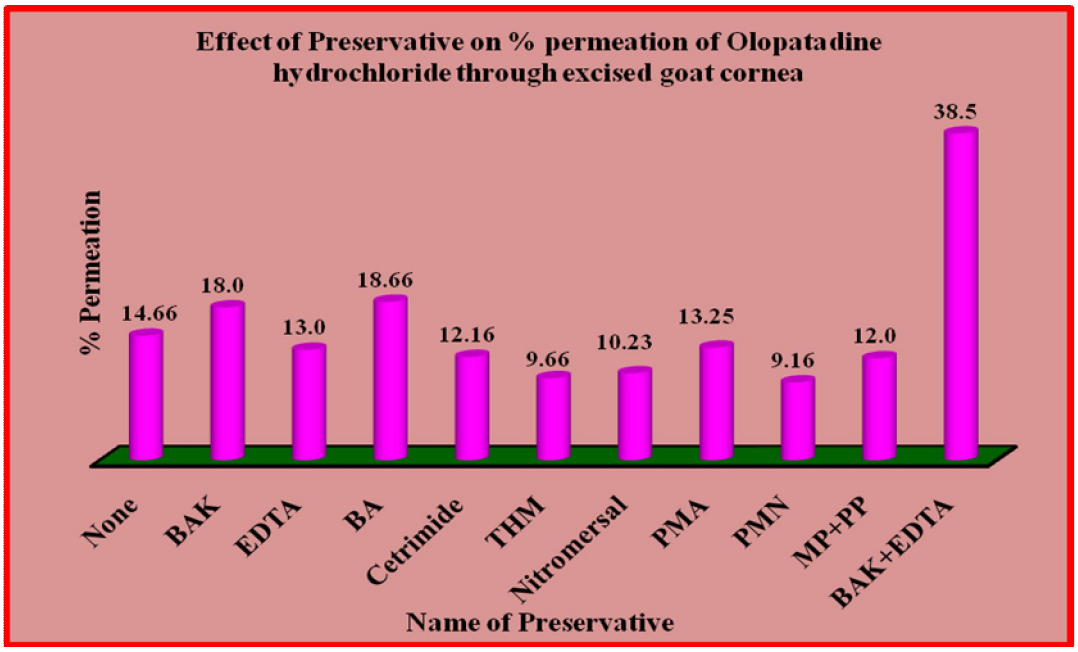

Figure 3 - Effect of Preservative on % permeation of Olopatadine hydrochloride

Table 4 - Effect of Preservative on the surface tension of Olopatadine hydrochloride (0.1%w/v, pH 7) ophthalmic Solution

Sl. No.	Preservative	Surface tension (dyne/cm)
1	None	70.87
2	Benzalkonium chloride	24.86
3	EDTA	45.27
4	Benzyl alcohol	48.10
5	Cetrimide	28.04
6	Thiomersal	56.35
7	Nitromersal	64.15
8	Phenyl mercuric acetate	59.92
9	Phenyl mercuric nitrate	57.60
10	Methyl paraben+ Propyl paraben	54.92
11	Benzalkonium Chloride + EDTA	36.20

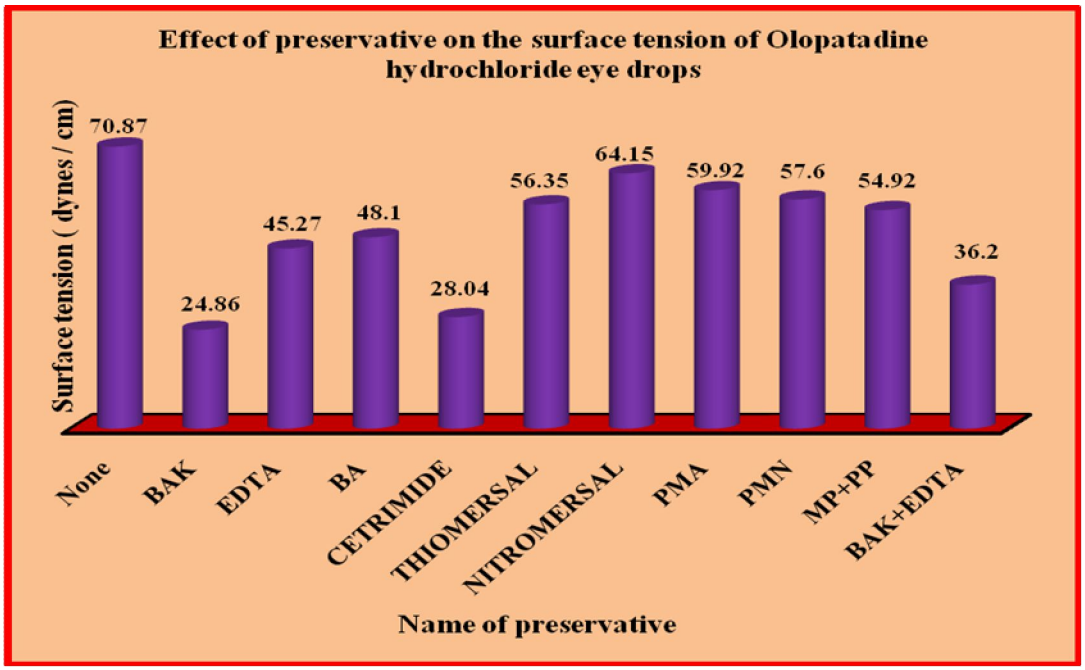

Figure 4- Effect of preservative on the surface tension of Olopatadine hydrochloride

Table 5 – Effect of formulation on partition coefficient of Drug

Sl. No.	Formulation	Conc. of drug in water (mg/ml)	Conc. of drug in Isobutanol (mg/ml)	Partition coefficient ($C_{w/\ Isobutanol}$)
1	EDTA	0.265	0.735	0.360
2	BAK+EDTA	0.265	0.735	0.360
3	Control	0.265	0.735	0.360

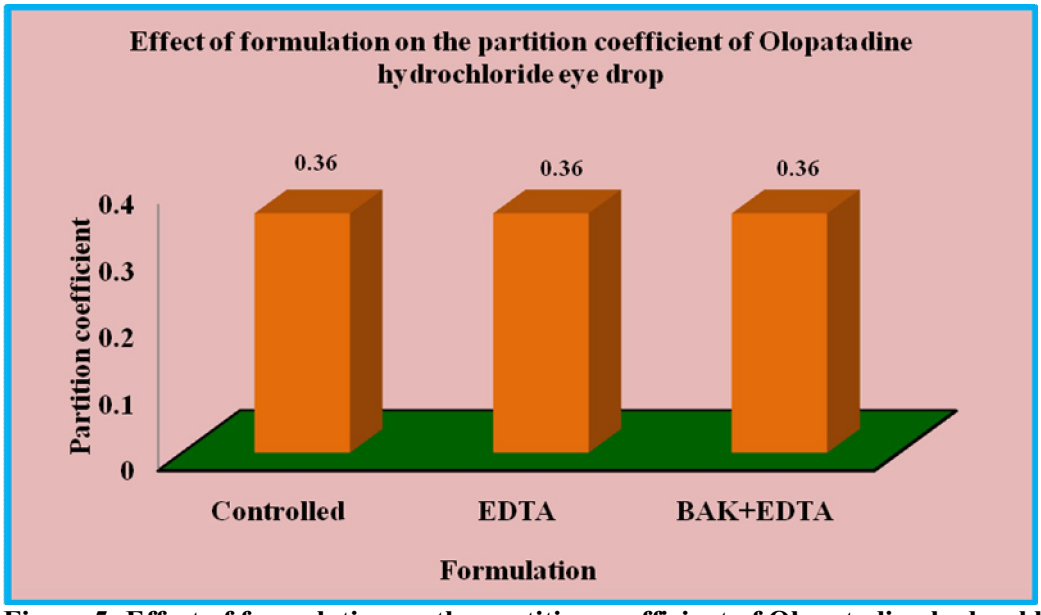

Figure 5- Effect of formulation on the partition coefficient of Olopatadine hydrochloride

Table 6 - Relative permeation characteristics of Olopatadine from Control, Optimized (BAK + EDTA), and Selected marketed formulations Through Excised Goat Cornea

Formulations	pH	Amount permeated (mg)	% Permeation	Corneal Hydration (%)	Surface Tension (dyne/cm)	0.1 N NaOH consumed (ml)
Control	7.0	0.145	14.5	78.37 ± 0.2	70.8	0.08
Optimized	7.0	0.385	38.5	77.85 ± 0.81	36.2	0.08
Olopine	6.97	0.363	36.33	77.85 ± 0.32	36.3	0.08
Olopat	7.0	0.346	34.66	79.80 ± 0.57	22.7	0.08

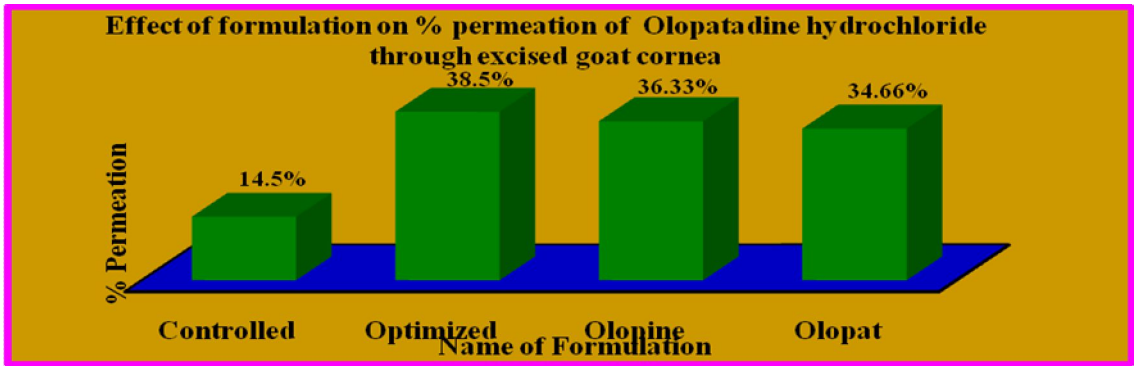

Figure 6- Effect of formulation on % permeation of Olopatadine hydrochloride

Table 7 - Stability Studies

Time (Days)	Colour	Particulate matter	pH	Drug Content (%)	Amount Permeated(mg)	% Permeation	Corneal Hydration (%)
0	Colourless	Not found	7.04 ± 0.0	102	0.385	38.5	77.85
7	Colourless	Not found	6.99±0.1	101.35	0.382	38.2	78.44
15	Colourless	Not found	6.97±0.08	101.18	0.380	38.0	78.87
30	Colourless	Not found	6.96±0.07	99	0.377	37.7	79.35
45	Colourless	Not found	6.96±0.07	98.5	0.376	37.6	79.53
60	colourless	Not found	6.96±0.07	98.4	0.376	37.6	79.56

CONCLUSIONS:

On the basis of present studies it can be concluded that increase in concentration of Olopatadine hydrochloride in aqueous drop causes a disproportionate increase in permeation. Corneal transport of Olopatadine hydrochloride is pH dependent, having maximum transport at physiological pH of tears (i.e., 7.0). Olopatadine hydrochloride, 0.1% w/v aqueous drop (pH 7.0), containing Benzalkonium chloride (BAK, 0.01% w/v) and EDTA (0.01% w/v) provides highest *in vitro* ocular availability through goat corneas among all the formulations including marketed products like olopine, olopat. The corneal hydration of all the formulations with respect to effect of concentration, pH and preservative was within the normal range of 75-80%, indicating that there is no corneal damage. There was decrease in surface tension of the optimized formulation, indicates the increase in corneal permeation by corneal disruption or by emulsification of corneal epithelium due to presence of BAK and EDTA.There was no buffer in the marketed product as well as in the optimized formulation.Among the marketed formulations, Olopine showed higher permeation than Olopat. The stability study also indicates the optimized formulation stable with highest permeability with respect to all other formulations.

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