
Formulation of the Model Fluconazole Eye Drop and Its Comparison with the Available Fluconazole Eye Drops

Sunil Thakral¹, Munish Ahuja²

¹Akal College of Pharmacy and Technical Education, Sangrur, India, ² Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar, India

Abstract

The aim of the study was to formulate the model formulation of fluconazole eye drop and then compare it with the available eye drops. The corneal permeation studies were conducted using freshly excised sheep cornea, mounted between donor and receptor. The receptor cell had an internal volume of 11 mL, containing ringer bicarbonate (pH 7.4, 34±1 °C). At appropriated intervals 2 ml. samples were withdrawn from the side arm and were analyzed spectrophotometrically by measuring absorbance at λ_{\max} of 260 nm. Each experiment was continued for about 2.0 hrs (triplicate). At the end of the experiment, each cornea (freed from sclera) was weighed, soaked in 1 mL. methanol, dried overnight at 90 °C and reweighed. From the difference in weights corneal hydration was calculated. Even though, the marketed formulation (Zocon) comprised of 0.3% w/v of fluconazole and our model formulation contained only 0.2% w/v of fluconazole, the amount of fluconazole permeated from model formulation and the marketed formulation was respectively 78.34±4.26 and 22.14±1.3. The permeation from model formulation was much greater than other preparations and shows less corneal hydration (80.29±0.47) than others available preparations.

Key words: Fungal keratitis, fluconazole, in vitro permeation, corneal hydration, model formulations

Formulasi Tetes Mata Fluconazole dan Perbandingannya dengan Tetes Mata Fluconazole di Pasaran

Abstrak

Penelitian ini bertujuan untuk melakukan formulasi tetes mata flukonazol dan membandingkannya dengan tetes mata yang beredar di pasaran. Studi permeasi kornea dilakukan dengan menggunakan kornea biri-biri yang telah dikeluarkan, disatukan antara donor dan reseptor. Sel reseptor memiliki volume internal 11 mL, mengandung ringer bicarbonate (pH 7,4, 34±1 °C). Sampel diambil pada interval 2 mL sampel dari bagian lengan bejana dan dianalisis menggunakan spektrofotometri dengan pengukuran absorbansi pada λ_{\max} 260 nm. Percobaan dilanjutkan selama dua jam (triplikasi). Pada akhir percobaan, setiap kornea (dipisahkan dari sklera) ditimbang, direndam dalam metanol, dikeringkan pada suhu 90 °C dan ditimbang ulang, perbedaan berat dari hidrasi kornea dihitung. Formulasi yang dipasarkan (Zocon) terdiri atas 0,3% w/v flukonazol sedangkan model formulasi dari penelitian ini hanya mengandung 0,2% w/v flukonazol, jumlah flukonazol yang mengalami permeasi dari model formulasi dan formulasi dipasarkan masing-masing sebesar 78,34±4,26 and 22,14±1,3. Permeasi dari model formulasi lebih besar dibandingkan dengan sediaan dan menunjukkan nilai hidrasi korneal lebih kecil (80,29±0,47) dibandingkan dengan sediaan di pasaran.

Kata kunci: Fungal keratitis, fluconazole, permeasi *in vitro* corneal hydration, model formulations

Corresponding author: Sunil Thakral, M.Pharm., Akal College of Pharmacy and Technical Education, Mastuana Sahib, Sangrur, Punjab, India, *email:* sunil.thakral@gmail.com

Introduction

Fungal eye infections are rare.¹ The number of fungal infections has increased dramatically, and those involving the eye pose a serious problem and treatment challenge to practicing physicians.² Fungal keratitis is a major blinding eye disease in Asia.³ This disease is quite common in the tropics and with large agrarian population.⁴ Fungal keratitis is a serious and painful corneal inflammation that results from infection by a fungal organism.⁵ The symptoms of fungal keratitis are blurred vision; a red and painful eye that does not improve when contact lenses are removed, increased sensitivity to light, and excessive tearing or discharge.⁴

A presumptive diagnosis of fungal keratitis requires immediate empirical therapy.⁴ The antifungal agents available today are merely fungistatic, and require an intact immune system and prolonged therapeutic course.^{3,6}

The synthetic biazole antifungal compound fluconazole exhibits outstanding physical and pharmacokinetic properties. Fluconazole is a stable, water-soluble, bis-triazole antifungal that has low molecular weight, high bioavailability, good ocular penetration when used either systemically or topically, and low toxicity. It is potentially useful as a topical ocular agent. It is quite effective against candida species.⁷ Fluconazole prevents the synthesis of ergosterol, a major component of fungal plasma membranes, by inhibiting the cytochrome P-450-dependent enzyme lanosterol demethylase (also referred to as 14 α -sterol demethylase or P-450DM). This enzyme also plays an important role in cholesterol synthesis in mammals.⁸

For the ailments of the eye, topical administration is usually preferred over systemic administration so as to avoid systemic toxicity, for rapid onset of action, and for decreasing the required dose. Though topical administration offers many advantages to treat disorders of anterior structures of the eye, it suffers from

a serious disadvantage of poor bioavailability due to several biological factors, which exist to protect the eye and consequently limit the entry of ocular drugs.⁹ Ocular conditions are usually treated by topical administration of drug solutions administered as eye drops into the lower cul-de-sac. These conventional dosage forms account for around 90% of the available ophthalmic formulations, mainly due to their simplicity and convenience.¹⁰

Drugs are commonly applied to the eye for a localized action. A major problem in ocular therapeutics is the attachment of an optimal drug concentration at the site of action. Poor bioavailability of drugs from ocular dosage forms is due to the precorneal loss factors, physiological and anatomical constraints.¹¹ Consequently, after instillation of eye drops, typically less than 5% of an applied dose reaches the intraocular tissues.¹² This forces the clinician to recommend a frequent dosing at an extremely high concentration and pulse type dosing results in several side effects of ophthalmic products.⁹ Several mechanisms such as a relatively impermeable corneal barrier and rapid drainage of the installed solution protect to the eye. Drugs are mainly estimated from the precorneal lachrymal fluid by solution drainage, lacrimation and nonproductive absorption of the conjunctiva of the eye. These factors and the corneal barrier limit the penetration of the topically administered drug into the eye.¹²

Methods

a. Materials

Fluconazole was obtained as a gift sample from APL research Center, Mandal (A.P.). Some marketed preparations of fluconazole eye drop such as Conflu (East India Pharmaceutical Works Ltd., Kolkata), Corneal (Ahlcon Parenterals (India) Ltd., Bhiwadi), Zocon (FDC Limited, Waluj) were procured from local pharmacy of Hisar, India. Hydroxy propyl methyl cellulose-E-50 LV

premium (HPMC) was purchased from Loba Chemie Pvt. Ltd., Bombay. Sodium chloride, potassium chloride, magnesium chloride, calcium chloride, sodium bicarbonate, sodium dihydrogen orthophosphate and mannitol were purchased from Qualigens fine chemicals (Mumbai, India). All other chemicals purchased were of analytical grade and were used as received. Fresh whole eye ball of sheep were obtained from local butcher shop (Hisar, India), within a half hour of slaughtering of the animal. The apparatus used in permeation studies was same as published elsewhere.¹³

b. Preparations of model formulation

The effects of different formulation parameters on corneal permeation were characterized and the model formulation of fluconazole was developed on the basis of the previous result.¹⁴

Fluconazole	-	0,2%
Benzyl alcohol	-	0,5%
HPMC	-	1,0%
Mannitol	-	2,88%
Phosphate buffer (pH 6.0)	-	q.s

The model formulation of fluconazole was prepared by dissolving the HPMC in phosphate buffer, then mannitol was added in this solution and then fluconazole and benzyl alcohol were mixed in above solution with continuous shaking on vortex shaker, and finally the volume was make up using phosphate buffer.

c. Method of analysis

Stock Solution

An accurately weighed 25 mg of fluconazole was dissolved in up to 100 ml. water, Ringer Bicarbonate (pH 7.4) and Sorenson phosphate (pH 7.4) in 100 ml. volumetric flasks (Class A) to give solution of 250 µg/ml.

Standard Solutions

Stock solutions (250µg/ml) of fluco-

nazole, as prepared above, were diluted to give 10, 25, 50, 100, 150, 200 and 250 µg/ml with appropriate dilution with water, Ringer Bicarbonate (pH 7.4) and Sorenson phosphate (pH 7.4). All the solutions were made in triplicate.

Preparation of standard curve

The standard curves were prepared by measuring the absorbance at 260 nm (λ_{max}) of standard solutions. The data so obtained was plotted, and regression was carried out.

d. In Vitro transcorneal permeation study¹⁵⁻¹⁷

Corneal preparation

Whole eye ball of the sheep was obtained from local butcher's shop within half an hour of slaughtering of the animal, and was transported to the laboratory in cold (4°C) normal saline (0.9%) immediately. The corneal was carefully excised along with 2–4 mm. of surrounding scleral tissues and washed with cold normal saline till free from proteins.

Permeation experiment

Fresh cornea was mounted by sandwiching the surrounding scleral tissue between clamped donor and receptor cells of modified version of Franz diffusion cell in such a way that its epithelial surface (apical) faced the donor compartment and endothelial surface faced to receptor compartment. Cell was placed on magnetic stirrer in holding position. The receptor compartment was filled with 11 ml. of freshly prepared bicarbonate ringer solution (pH 7.4) and stirred using Teflon coated magnetic stir bar. Drug solution (1 ml.) was placed to the epithelial side of cornea in donor cell and stirring of the receptor fluid (jacketed with water at $34\pm 1^\circ\text{C}$) was started. At appropriated intervals 2 ml. samples were withdrawn from the receptor compartment and withdrawn sample volume was replaced with equal volume of fresh bicarbonate ringer solution to ensure sink conditions. Withdrawn

samples were analyzed using Varian-Cary 5000 UV-VIS-NIR spectrophotometer by measuring absorbance at λ_{max} . of 260 nm. Each experiment was continued for about 2.0 hrs and was performed at least in triplicate.

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

Calculation of Apparent permeability coefficient

The apparent permeability coefficient was calculated using following equation:

$$P_{app} = \Delta Q / \Delta t \times 1 / (A.C_0.60)$$

Where $\Delta Q / \Delta t$ ($\mu\text{g}/\text{min.}$) is the flux across corneal tissue, A is the exposed surface area of corneal tissue (0.786 cm^2), C₀ is the initial drug concentration ($\mu\text{g}/\text{ml.}$) in the donor compartment and 60 is included to convert minutes to seconds.

Results

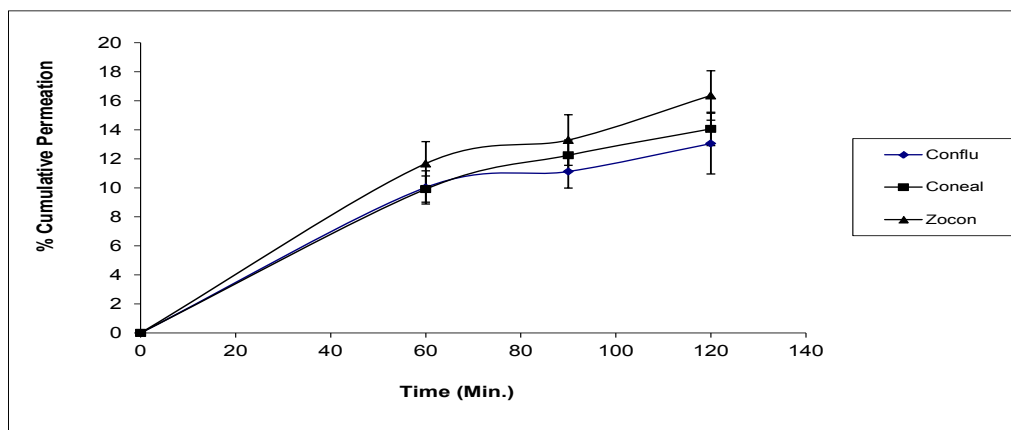


Figure 1 Comparative in vitro transcorneal permeation of fluconazole from commercial eye drops

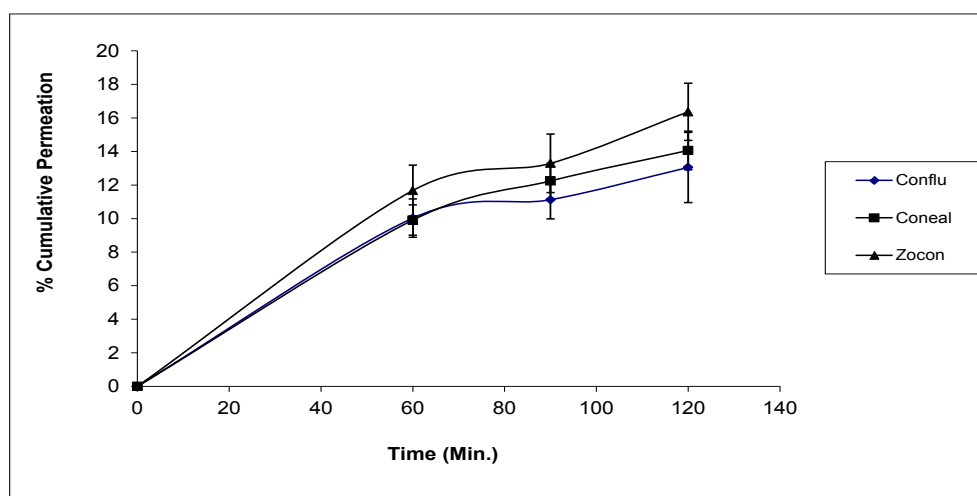


Figure 2 In vitro transcorneal permeation of fluconazole from model fluconazole eye drops

Tabel 1 Comparative corneal permeation of fluconazole from commercial eye drops

Eye drops	pH	Surface tension (dyne/cm.)	Viscosity (cps)	% cumulative Permeation*	$P_{app} \times 10^7$ (cm./sec)	Relative P_{app}	Titre Value (ml.)	% Corneal Hydration		
				60	90	120				
Conflu	4.0	37.09±1.21	6.1042±0.3	6.10.03±1.1	611.13±1.14	13.05±2.10	7.78±1.17	0.92	4.6	83.24±0.93
Corneal	6.1	40.94±1.38	1.038±0.34	9.91±0.91	12.25±1.06	14.06±1.05	8.50±0.73	1.00	0.358	83.05±1.49
Zocon	6.0	34.29±2.89	1.026±0.22	11.67±1.52	13.29±1.75	16.37±1.70	9.05±1.06	1.06	0.316	82.41±0.21

*Values are mean±S.D. (n=3).

Tabel 2 Comparative corneal permeation of fluconazole from commercial eye drops

Eye drops	pH	Surface tension (dyne/cm.)	Viscosity (cps)	% cumulative Permeation*	$P_{app} \times 10^6$ (cm./sec)	% Corneal Hydration		
				60	90	120		
Model	6.0	40.42±0.07	8.38±0.25	53.32±5.31	67.092±5.15	78.34±4.26	7.09±0.37†	80.29±0.47
Zocon	6.0	34.29±2.89	1.026±0.21	14.53±1.94	17.62±1.44	22.14±1.35	1.31±0.08	82.04±0.82

*Values are mean±S.D. (n=3). † Significantly different (p < 0.05) as compared to control as determined by paired t-test

The flux across the cornea was determined from the slope of the regression line obtained from the linear part of the curve between the cumulative amount permeated (Q) vs. time (t) plot.

Statistical analysis

Statistical calculation were done by one-way analysis of variance (ANOVA) followed by Dunnett's test. A p value <0.05 was considered significant.

Discussion

Table 1 and Figure 1 compare the marketed eye drops. The results of in vitro corneal permeation study show that the permeation of fluconazole from various marketed formulations follows the order Zocon > Corneal > Conflu. However no significant difference was observed between the apparent corneal permeability of different marketed formulations. The normal cornea has a hydration level of 75-80%. The maximum corneal hydration level attainable without producing irreversible damage to tissue is 83%. All the marketed formulations had a corneal hydration of > 82 %, suggesting slight corneal damaging potential. Table 2 and Figure 2 showing the comparison of the corneal permeation of model and marketed fluconazole eye drops. Eventhough, the marketed formulation (Zocon) comprised of 0.3% w/v of fluconazole and our model formulation contained only 0.2% w/v of fluconazole, the amount of fluconazole permeated from model formulation was much greater than the marketed formulation. The model formulation was prepared using some other additives which were proved to enhance the invitro corneal permeation.

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Conclusions

In recent years, exclusive investigations have been dedicated to prolonging the retention time of medications in the eye surface and to the improvement of transcorneal penetration of traditional and of novel therapeutic agents. By using polymers we can enhance the residential time and by using permeation enhancers and some other additive which affect corneal permeation we can also increase the in vitro corneal permeation.

On the basis of transcorneal permeation studies, an optimal dosage form containing fluconazole (0.2% w/v) in a Sorenson phosphate (0.0667 M, pH 6.0) buffered vehicle, mannitol (2.88%), benzyl alcohol (0.5% w/v), hydroxyl propyl methyl cellulose (1.0% w/v) was formulated. On comparison of fluconazole permeation from optimized model formulation with a marketed eye drop (Zocon, F.D.C. Limited) through paired sheep cornea, a significant enhancement of in vitro corneal permeation from model formulation was observed. By enhancing the invitro corneal permeation dosing frequency of eye drops can

be reduced and concentration of the active ingredient can also be decreased with better results. However, further investigations in in vivo model are needed to assess the ocular bioavailability of in vitro optimized model formulation.

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