Pre-formulation study on API characterization of Brimonidine Tartrate, Timolol maleate and Dorzolamide Hydrochloride in Anti-glaucoma drugs.

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ABSTRACT

The aim of this study was to develop the Glaucoma drug. In addition, a preformulation study and physical properties of the finished products were investigated to select the best formulation for further study. Pre-formulation studies are evaluated by the physical and chemical properties of the active pharmaceutical ingredient (API), Assay values by non aqueous titration and HPLC method and IR spectrum by using Fourier transformer infrared spectroscopy. The knowledge gained on the API helps to select the right salt or polymorphic form, and supports the design and development of stable as well as therapeutically effective and safe dosage form. HPLC method for identification of Brimonidine tartrate, Timolol Maleate and Dorzolamide HCl at wavelength from 4000cm to 400cm1. The assay values are obtained by non aqueous titration for Brimonidine tartrate, Timolol Maleate is 99.70% and 100.18% and Dorzolamide HCl in HPLC method the value is 100.73%. The overall objective of pre-formulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms which can be mass-produced. In present research work characterizes the Glaucoma drug by its characterization of the API (Brimonidine tartrate, Timolol maleate and Dorzolamide Hydrochloride) during pre-formulation which includes determination of: description, Solubility profile, identification by Infrared and HPLC and Assay.

Keywords: Brimonidine tartrate, Timolol Maleate, Dorzolamide Hydrochloride, Glaucoma, Intraocular pressure, Pre-formulation, Active Pharmaceutical Ingredients (API).
INTRODUCTION

Glaucoma is a group of ocular disorders with multi-factorial etiology united by a clinically characteristic intraocular pressure-associated optic neuropathy [1]. This can permanently damage vision in the affected eye(s) and lead to blindness if left untreated. It is normally associated with increased fluid pressure in the eye (aqueous humour) [2]. The term "ocular hypertension" is used for people with consistently raised intraocular pressure (IOP) without any associated optic nerve damage.

API CHARACTERIZATION

Brimonidine tartrate

Brimonidine tartrate is a quinoxaline derivative and adrenergic alpha-2 receptor agonist that is used to manage intraocular pressure associated with open-angle glaucoma and ocular hypertension.

Brimonidine Tartrate is the tartrate salt form of brimonidine, an imidazole derivative and a selective alpha-2 adrenergic receptor agonist. Upon ocular administration, brimonidine tartrate acts on the blood vessels causing them to constrict which leads to a decrease in the production of aqueous humor. Brimonidine tartrate also enhances the outflow of aqueous humor. This drug is used in the treatment of glaucoma to reduce intraocular pressure [3].

Chemical name:
5-Bromo-N-(imidazolidin-2-ylidene) quinoxalin-6-amine(2R,3R)-2,3-dihydroxybutanedioate

Chemical Formula: C_{15}H_{16}BrN_{5}O_{6}

Chemical structure

Molecular weight: 442.2
Appearance
White or slightly yellowish or slightly brownish powder
Solubility
Soluble in water, practically insoluble in anhydrous ethanol and in toluene
ASSAY
99.0% - 101%
Dissolve 0.350 g in 70 mL of anhydrous acetic acid R using sonication until complete dissolution. Titrate with 0.1 M perchloric acid, determining the endpoint potentiometrically
1 mL of 0.1 M perchloric acid is equivalent to 44.22 mg of C_{15}H_{16}BrN_{5}O_{6}(Table 1).

Timolol Maleate

Chemical name:
(2R)-1-[(2-Methyl-2-propanyl)amino]-3-[[4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl]oxy]-2-propanol (2E)-2-butenedioate (1:1)

Chemical Formula: C_{13}H_{24}N_{4}O_{3}.C_{4}H_{4}O_{4}

Chemical structure
Molecular weight: 432.49

**Appearance**
White or almost white crystalline powder

**Solubility**
Soluble in water

**ASSAY**
98.5% - 101%

Accurately weighed about 0.35gm of the substance dissolve in 60ml of anhydrous glacial acetic acid. Titrate with 0.1M perchloric acid determine the end point is potentiometrically. Carry out a blank titration. Each ml of 0.1M Perchloric acid is equivalent to 0.04325gm of timolol maleate [4,5] (Table 1).

**Dorzolamide Hydrochloride**

**Chemical name:** 4S, 6S)-4-(Ethylamino)-6-methyl-5,6-dihydro-4H-thieno[2,3-b]thiopyran-2-sulfonamide 7,7-dioxidehydrochloride (1:1

**Chemical Formula:** C_{10}H_{16}N_{2}O_{4}S_{3}, HCl

**Chemical structure**

![Chemical structure of Dorzolamide Hydrochloride]

**Molecular weight:** 360.901

**Appearance**
White or almost white crystalline powder

**Solubility**
Soluble in water.

**ASSAY BY HPLC**
99.0% - 101%

**Mobile phase Preparation:** Buffer: Methanol (93.5:6.50)

Buffer preparation: 3.70 gm of Potassium dihydrogen ortho phosphate in 1000ml with water.

**Standard preparation**
: Weigh accurately 220 mg of Dorzolamide Hydrochloride RS and is diluted to 50 ml with mobile phase. Take 2 ml of this solution is further diluted to 10 ml with mobile phase.
Sample preparation: Weigh accurately 220 mg of Dorzolamide Hydrochloride and is diluted to 50 ml with mobile phase. Take 2 ml of this solution is further diluted to 10 ml with mobile phase. (Table 1)

Chromatographic condition: Column - 4.6mm x 25cm
Flow rate - 1.50 ml / min
Wave length - 254 nm
Loop size - 20µl
Temperature- Ambient

Table 1: Specification of BTD (Brimonidine tartrate, Timolol Maleate, Dorzolamide Hcl)

<table>
<thead>
<tr>
<th>Specification</th>
<th>Brimonidine tartrate</th>
<th>Timolol Maleate</th>
<th>Dorzolamide Hydrochloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>A white to slightly yellowish crystalline powder</td>
<td>A white or almost white crystalline powder</td>
<td>White to off- white, crystalline powder</td>
</tr>
<tr>
<td>Solubility</td>
<td>Soluble in water</td>
<td>Soluble in water</td>
<td>Soluble in water</td>
</tr>
<tr>
<td>IR identification test</td>
<td>IR spectrum of sample corresponds to that of standard spectrum</td>
<td>IR spectrum of sample corresponds to that of standard spectrum</td>
<td>IR spectrum of sample corresponds to that of standard spectrum</td>
</tr>
<tr>
<td>HPLC identification test</td>
<td>The retention time for sample peak corresponds to that of standard peak</td>
<td>The retention time for sample peak corresponds to that of standard peak</td>
<td>The retention time for sample peak corresponds to that of standard peak</td>
</tr>
<tr>
<td>Assay</td>
<td>98.0% - 102.0%</td>
<td>98.5% - 101.0%</td>
<td>99.0% - 101%</td>
</tr>
</tbody>
</table>

PHARMACOLOGY

Brimonidine tartrate is a potent and selective agonist of alpha-2 adrenergic receptor has an affinity 1000 times greater for the alpha-2 receptor than for the alpha receptor 1. It is highly lipophilic main route of ocular penetration after topical administration is through the cornea. It seems to have a much lower allergic response associated with it and is much more effective as chronic therapy for most patients.

Timolol Maleate is a beta blocker agent onset of action with the drop can be detected within first hour with the maximum effect observed at 2-4 hours. They lower Intra Ocular Pressure by decreasing the rate of aqueous production.

Dorzolamide is a Carbonic anhydride inhibitors IOP is lowered by a direct action on the ciliary epithelium to suppress the secretion of aqueous humor inflow. Carbonic anhydrase inhibitors are often used as adjunctive therapy.

The combined formulation results may give greater decrease in IOP than that achieved with either component alone[6].

PHARMACOKINETICS

Dorzolamide hydrochloride is a topical carbonic anhydrase II inhibitor and timolol maleate is a topical beta-adrenergic receptor blocking agent. In combination, they are approved to reduce elevated IOP in patients with open-angle glaucoma or ocular hypertension and with insufficient IOP response to beta-blockers monotherapy.

Both Brimonidine, dorzolamide and timolol help reduce IOP by decreasing the production of aqueous humor by the ciliary body. Carbonic anhydrase inhibition slows the formation of bicarbonate ions thereby decreasing the amount of sodium and fluid transport. With such a decrease in fluid transport comes a decreased production of aqueous humor. Dorzolamide decreases the secretion of aqueous humor in the ciliary processes by inhibition of carbonic anhydrase II, the most active isoenzyme and found primarily in red blood cells. Thus, chronic administration of dorzolamide causes an accumulation of the medication within red blood cells. This drug also binds moderately to plasma proteins. Metabolism of dorzolamide produces N-desthyl...
which also binds to red blood cells to inhibit carbonic anhydrase I to a greater extent than carbonic anhydrase II. The major route of excretion is through the urine for both the parent and metabolite drug. Upon discontinuation of the medication there is a rapid initial decline of the medicine from the red blood cells followed by a much slower decline due to an elimination-phase half-life of approximately 4 months. Carbonic anhydrase inhibitor has been reported to increase ocular blood flow parameters by causing ocular vasodilation through metabolic acidosis via elevated carbon dioxide levels in the eye tissues in normal tension glaucoma patients A high concentration of topically applied dorzolamide has been shown to reach the choroid of the posterior pole of the eye. It has been a popular adjunctive agent and is often used as monotherapy. Dorzolamide is also a safer alternative to the oral carbonic anhydrase inhibitor, acetazolamide and methazolamide, in the treatment of primary open-angle glaucoma or ocular hypertension. Dorzolamide reduces IOP from baseline at trough by 15%–19% and at peak by 20%–24%.

Timolol is a non-selective beta-adrenergic antagonist. Reducing aqueous humor flow is the main mechanism by which beta blockers like timolol have been shown to lower IOP. Timolol presumably exerts a direct action on the beta-2 adrenergic receptors in the ciliary processes to decrease aqueous humor secretion and possibly on local capillary perfusion to reduce ultrafiltration Reduction of aqueous humor production may be secondary to inhibition of catecholamine-stimulated synthesis of cyclic adenosine monophosphate (AMP) in ciliary epithelium, which has been demonstrated in rabbit studies. However, the regulation of aqueous humor dynamics is complex and still not fully understood. Studies have shown a topical timolol effect on aqueous flow in the fellow, untreated eye in patients with open-angle glaucoma and with ocular hypertension Timolol decreases IOP by approximately 20%–30%[7,8]

PREFORMULATION STUDY

Identification of drug by FTIR method

Fourier Transform Infrared analysis of drugs:

The FTIR analysis of the API Brimonidine tartrate, Timolol maleate and Dorzolamide Hydrochloride was carried out for qualitative compound identification. The KBr pellet of approximately 10mm diameter of the drug was prepared grinding 10mg of sample with 1gm of KBr in pressure compression machine. The infraspectrum of levofloxacin in a KBr pellet for wavenumber range of 4000 – 500cm⁻¹

Identification of drug by HPLC method

High Performance Liquid Chromatography: The retention time of the sample peak should corresponds with that of peak obtained with standard solution [9-12].

Brimonidine tartrate, Timolol Maleate and Dorzolamide Hydrochloride

Mobile phase Preparation : Buffer: Methanol (93.5:6.50)
Buffer Preparation : 3.70 gm of Potassium dihydrogen ortho phosphate in 1000ml with water.
Standard preparation: Weigh accurately 220 mg of Dorzolamide Hydrochloride RS, 50mg of Timolol Maleate RS and 20 mg of Brimonidine tartrate RS into 50ml SMF and dissolved in mobile phase and diluted upto 50 ml with mobile phase. Take 2 ml of this solution is diluted to 10 ml with mobile phase.
Sample preparation: Weigh accurately 220 mg of Dorzolamide Hydrochloride RS, 50mg of Timolol Maleate RS and 20 mg of Brimonidine tartrate RS into 50ml SMF and dissolved in mobile phase and diluted upto 50 ml with mobile phase. Take 2 ml of this solution is diluted to 10 ml with mobile phase.

Chromatographic condition : Column - 4.6mm x 25cm
Flow rate - 1.50 ml / min
Wave length - 254 nm
Loop size - 20µl
Temperature- Ambient

Assay of Brimonidine tartrate by Non-aqueous solution
99.0% - 101.0%
Dissolve 0.350 g in 70 mL of anhydrous acetic acid R using sonication until complete dissolution. Titrate with 0.1 M perchloric acid, determining the endpoint potentiometrically 1 mL of 0.1 M perchloric acid is equivalent to 44.22 mg of C15H16BrN5O6.

**Assay of Timolol Maleate by Non-aqueous solution**

98.5% - 101%

Accurately weighed about 0.35 gm of the substance dissolve in 60 ml of anhydrous glacial acetic acid. Titrate with 0.1M perchloric acid determine the end point is potentiometrically. Carry out a blank titration. Each ml of 0.1M Perchloric acid is equivalent to 0.04325 gm of timolol maleate.

**Assay of Dorzolamide HCl by HPLC method**

99.0% - 101%

Mobile phase Preparation: Buffer: Methanol (93.5:6.50)

Buffer preparation: 3.70 gm of Potassium dihydrogen ortho phosphate in 1000 ml with water.

Standard preparation: Weigh accurately 220 mg of Dorzolamide Hydrochloride RS and is diluted to 50 ml with mobile phase. Take 2 ml of this solution is further diluted to 10 ml with mobile phase.

Sample preparation: Weigh accurately 220 mg of Dorzolamide Hydrochloride and is diluted to 50 ml with mobile phase. Take 2 ml of this solution is further diluted to 10 ml with mobile phase.

Chromatographic condition: Column - 4.6mm x 25cm
Flow rate - 1.50 ml/min
Wave length - 254 nm
Loop size - 20µl
Temperature- Ambient

**Solubility determination**

Weighed 1 gm of Brimonidine tartrate, Timolol maleate, and Dorzolamide Hydrochloride into an individual boiling tube and dissolved in Purified water.

Each ingredient should be completed dissolved within 10 – 20 ml of Purified water.

**RESULTS AND DISCUSSION**

**Organoleptic Properties:**

Examine the Color, Crystalinity, Hygroscopicity, and odor of each ingredient of Brimonidine tartrate, Timolol maleate and Dorzolamide Hydrochloride through visual inspection (Table 2).

<table>
<thead>
<tr>
<th>Organoleptic Properties</th>
<th>Brimonidine tartrate</th>
<th>Timolol Maleate</th>
<th>Dorzolamide Hydrochloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Pale yellow</td>
<td>White</td>
<td>White</td>
</tr>
<tr>
<td>Crystalinity</td>
<td>Crystalline powder</td>
<td>Crystalline powder</td>
<td>Crystalline powder</td>
</tr>
<tr>
<td>Hygroscopicity</td>
<td>No Hygroscopicity</td>
<td>No Hygroscopicity</td>
<td>No Hygroscopicity</td>
</tr>
<tr>
<td>Odour</td>
<td>Odorless</td>
<td>Odorless</td>
<td>Odorless</td>
</tr>
</tbody>
</table>

**Identification test results**

**FTIR study for identification of Brimonidine tartrate, Timolol Maleate and Dorzolamide HCl:**

An FT infrared spectroscopy study was carried out to check the identity of sample spectrum (Figure 4,5,6) compatible with reference spectrum (Figure 1,2,3). The spectra obtained from Fourier transform infrared spectroscopy studies at wavelength from 4000 cm to 400 cm⁻¹.

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Figure 1: Timolol Maleate Standard IR spectrum

Figure 2: Brimonidine tartrate Standard spectrum
Figure 3: Dorzolamide Hydrochloride Standard spectrum

Figure 4: Timolol Maleate sample IR spectrum
IDENTIFICATION TEST RESULTS BY HPLC METHOD

HPLC study for identification of Brimonidine tartrate, Timolol Maleate and Dorzolamide HCl:

An HPLC study was carried out to check the identity of retention time for principal peak obtained with sample solution should corresponds with peak obtained with reference solution. The peak obtained from HPLC studies at wavelength of 254nm.
Assay of Timolol Maleate by Non-aqueous titration: Limit: 98.5% - 101%

Titre value for Timolol Maleate = 8.1 ml
Strength of 0.1M Perchloric acid = 1.0012
Weight of Timolol Maleate = 0.3501 gm

Assay of Timolol Maleate

\[ \text{Result} = \frac{\text{Titre value} \times \text{strength of 0.1M Perchloric acid} \times \text{Factor} \times 100}{\text{Weight of substance}} \]

\[ \text{Result} = \frac{8.1 \times 1.0012 \times 0.04325 \times 100}{0.3501} = 100.18\% \]

Assay of Brimonidine tartrate by Non-aqueous titration: Limit: 99.0% - 101%

Titre value for Brimonidine tartrate = 7.9 ml
Strength of 0.1M Perchloric acid = 1.0012
Weight of Timolol Maleate = 0.3508 gm

Assay of Brimonidine tartrate

\[ \text{Result} = \frac{\text{Titre value} \times \text{strength of 0.1M Perchloric acid} \times \text{Factor} \times 100}{\text{Weight of substance}} \]

\[ \text{Result} = \frac{7.9 \times 1.0012 \times 0.04422 \times 100}{0.3508} = 99.70\% \]

Assay of Dorzolamide Hydrochloride by HPLC: Limit: 99.0% - 101%

Sample Information

<table>
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<th>STD 2 Acquired by</th>
<th>Admin</th>
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<tbody>
<tr>
<td>Sample Name</td>
<td>Dorzolamide standard</td>
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<tr>
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</tr>
<tr>
<td>Tray#</td>
<td>1</td>
</tr>
<tr>
<td>Vault#</td>
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<tr>
<td>Injection Volume</td>
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<td>Data Filename</td>
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<td>Method Filename</td>
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</table>

Chromatogram

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<th>Height</th>
<th>Height %</th>
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<td>416552</td>
<td>100.000</td>
</tr>
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<td>Total</td>
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<td>Resolution</td>
<td>0.000</td>
<td>0.782</td>
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<td></td>
</tr>
</tbody>
</table>

PeakTable

PDA Ch 1 254nm 4nm
DISCUSSION

Preformulation is a group of studies that focus on the physicochemical properties of a new drug candidate that could affect the drug performance and the development of a dosage form. This could provide important information for formulation design or support the need for molecular modification. Every drug has intrinsic chemical and physical properties which have been considered before development of a pharmaceutical formulation[13,14]. This property provides the framework for drugs combination with pharmaceutical ingredients in the fabrication of dosage form. Objective of preformulation study is to develop the elegant, stable, effective and safe dosage form by establishing kinetic rate profile, compatibility with the other ingredients and establish Physico-chemical parameter of new drug substances. Among these properties, drug solubility, identification and assay are plays important role in pre-formulation study.

Hence we started the pre-formulation study for BTD formulation and assess the characterization of the ingredients of Brimonidine tartrate, Timolol Maleate and Dorzolamide HCl for its description, solubility, identification and assay.

CONCLUSION

For this study we were assessed physiochemical properties like description, solubility, identification and assay of Brimonidine tartrate, Timolol maleate and Dorzolamide HCl. Description of each material is
needed to identify all the solid forms that may exist as a consequence of the synthetic stage such as the presence of polymorphs. Solubility analysis of each ingredient of drug must possess some aqueous solubility for therapeutic efficacy. In order for a drug to enter the systemic circulation to exert a therapeutic effect, it must first be in solution. Relatively insoluble compounds often exhibit incomplete absorption. When a solute dissolves, the substance’s inter molecular forces of attraction must be overcome by forces of attraction between solute and solvent molecules. Identification test results by FTIR and HPLC will be helpful to assess compatibility of the material with drug formulation in qualitatively good. Assay or purity test results will give the drug formulation with safe and effective.

The data obtained in present study will be helpful in the formulation of anti-glaucoma drugs in fixed dose combination.

REFERENCES