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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).

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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

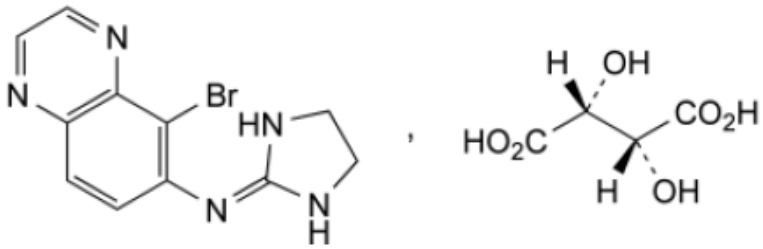
Brimonidine tartrate is a quinoxaline derivative and adrenergic alhpa-2 receptor agonist that is used to manage intraocular pressure associated with open-angle glaucoma and ocular hypertension.

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: $C_{15}H_{16}BrN_5O_6$

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 C15H16BrN5O6(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: $C_{13}H_{24}N_4O_3S.C_4H_4O_4$
Chemical structure

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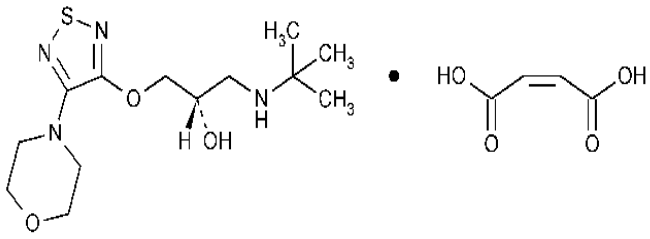


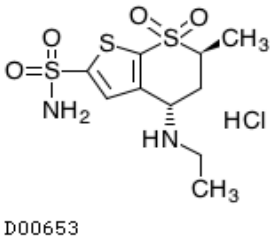
Molecular weight: 432.49

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: $C_{10}H_{16}N_2O_4S_3$. HCl
Chemical structure



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.

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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)

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

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 liphophilic 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 anhydrase 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].

PHARMOCOKINETICS

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 0.35gm 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.

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 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.
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 1gm 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 – 20ml of Purified water.

RESULTS AND DISCUSSION

Organoleptic Properties:

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

Table 2: Organoleptic Properties of Brimonidine tartrate, Timolol Maleate and Dorzolamide HCl

Organoleptic Properties	Brimonidine tartrate	Timolol Maleate	Dorzolamide Hydrochloride
Colour	Pale yellow	White	White
Crystalinity	Crystalline powder	Crystalline powder	Crystalline powder
Hygroscopicity	No Hygroscopicity	No Hygroscopicity	No Hygroscopicity
Odour	Odorless	Odorless	Odorless

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 4000cm to 400cm^{-1}

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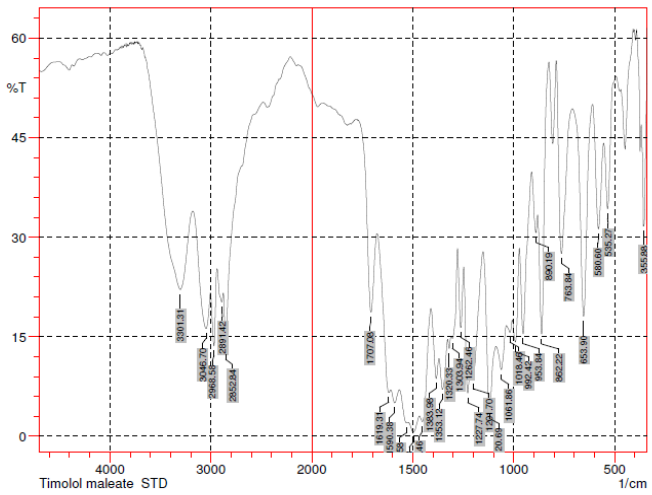


	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	355.88	31.7072	1.7329	356.85	339.49	6.5014	0.4871
2	535.27	34.2671	13.1548	554.56	494.76	21.1289	2.7702
3	580.6	31.2764	15.6066	609.53	555.52	22.2509	4.5161
4	653.9	18.0529	31.678	708.87	610.5	43.4624	13.5867
5	763.84	27.537	26.7692	788.92	709.83	32.1126	10.1878
6	862.22	15.3766	26.0643	881.51	826.53	27.4035	7.723
7	890.19	30.6803	4.4052	911.4	882.47	13.5969	0.888
8	953.84	15.4481	16.4261	972.16	912.37	35.5317	7.284
9	992.42	14.2014	7.9341	1006.89	973.13	25.3414	3.4191
10	1018.46	15.6387	1.5563	1035.82	1007.85	22.0414	0.5909
11	1061.86	10.0514	5.1086	1087.9	1036.78	46.0708	4.0752
12	1120.69	4.1627	16.6425	1151.55	1088.86	60.031	15.3014
13	1201.7	8.6416	5.6177	1213.28	1152.52	49.2187	3.8487
14	1227.74	6.5351	10.4405	1247.03	1214.24	31.7351	6.1342
15	1262.46	16.3797	10.4362	1277.9	1248	20.4118	3.3058
16	1303.94	14.7378	2.7954	1309.72	1278.86	23.0097	1.9988
17	1320.33	13.2125	1.5804	1327.08	1310.69	13.9639	0.3786
18	1353.12	7.0651	5.7896	1369.52	1328.05	42.1578	5.57
19	1383.98	8.6941	5.6059	1410.02	1370.48	36.0675	3.4493
20	1452.46	2.1934	5.0523	1466.93	1410.99	68.3076	7.2181
21	1496.83	0.6259	1.9337	1523.83	1467.89	103.556	14.3348
22	1530.58	2.0121	0.7754	1566.27	1524.79	60.2698	1.9058
23	1590.38	5.0398	1.9545	1608.7	1567.23	50.7969	2.8926
24	1619.31	6.6115	3.6964	1678.14	1609.67	56.6133	2.0727
25	1707.08	18.7111	17.0859	1769.76	1679.11	45.4072	7.8205
26	2852.84	13.0708	10.9702	2875.99	2693.71	99.5615	7.1168
27	2891.42	20.24	2.2517	2937.71	2876.95	40.3063	1.9192
28	2968.58	13.605	10.0017	3004.26	2938.68	48.4033	7.0377
29	3046.7	16.2343	8.3171	3178.83	3005.22	113.2108	14.8189
30	3301.31	22.1275	17.8372	3647.55	3179.79	222.8717	56.1365

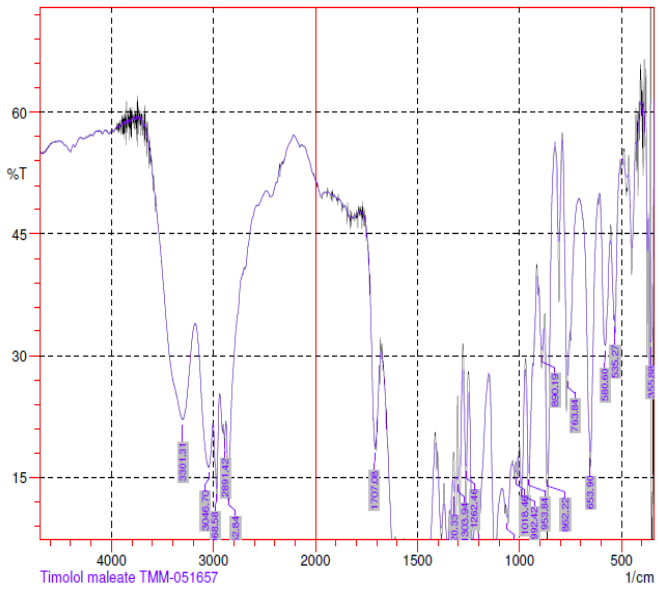
Date/Time:03/02/2016

Sample Name: Timolol Maleate standard.spc

Apodization;

Resolution;

Figure 1: Timolol Maleate Standard IR spectrum



	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1							
	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Area
1	355.88	31.7072	1.7329	356.85	339.49	6.5014	0.4871
2	535.27	34.2671	13.1548	554.56	494.76	21.1289	2.7702
3	580.6	31.2764	15.6066	609.53	555.52	22.2509	4.5161
4	653.9	18.0529	31.678	708.87	610.5	43.4624	13.5867
5	763.84	27.537	26.7692	788.92	709.83	32.1126	10.1878
6	862.22	15.3766	26.0643	881.51	826.53	27.4035	7.723
7	890.19	30.6803	4.4052	911.4	882.47	13.5969	0.888
8	953.84	15.4481	16.4261	972.16	912.37	35.5317	7.284
9	992.42	14.2014	7.9341	1006.89	973.13	25.3414	3.4191
10	1018.46	15.6387	1.5563	1035.82	1007.85	22.0414	0.5909
11	1061.86	10.0514	5.1086	1087.9	1036.78	46.0708	4.0752
12	1120.69	4.1627	16.6425	1151.55	1088.86	60.031	15.3014
13	1201.7	8.6416	5.6177	1213.28	1152.52	49.2187	3.8487
14	1227.74	6.5351	10.4405	1247.03	1214.24	31.7351	6.1342
15	1262.46	16.3797	10.4362	1277.9	1248	20.4118	3.3058
16	1303.94	14.7378	2.7954	1309.72	1278.86	23.0097	1.9988
17	1320.33	13.2125	1.5804	1327.08	1310.69	13.9639	0.3786
18	1353.12	7.0651	5.7896	1369.52	1328.05	42.1578	5.57
19	1383.98	8.6941	5.6059	1410.02	1370.48	36.0675	3.4493
20	1452.46	2.1934	5.0523	1466.93	1410.99	68.3076	7.2181
21	1496.83	0.6259	1.9337	1523.83	1467.89	103.556	14.3348
22	1530.58	2.0121	0.7754	1566.27	1524.79	60.2698	1.9058
23	1590.38	5.0398	1.9545	1608.7	1567.23	50.7969	2.8926
24	1619.31	6.6115	3.6964	1678.14	1609.67	56.6133	2.0727
25	1707.08	18.7111	17.0859	1769.76	1679.11	45.4072	7.8205
26	2852.84	13.0708	10.9702	2875.99	2693.71	99.5615	7.1168
27	2891.42	20.24	2.2517	2937.71	2876.95	40.3063	1.9192
28	2968.58	13.605	10.0017	3004.26	2938.68	48.4033	7.0377
29	3046.7	16.2343	8.3171	3178.83	3005.22	113.2108	14.8189
30	3301.31	22.1275	17.8372	3647.55	3179.79	222.8717	56.1365

Date/Time:03/02/2016

Sample Name: Timolol Maleate Sample TMM 051657.spc

Apodization; Happ-Genzel

Resolution; 2 [1/cm]

Figure 2: Brimonidine tartrate Standard spectrum

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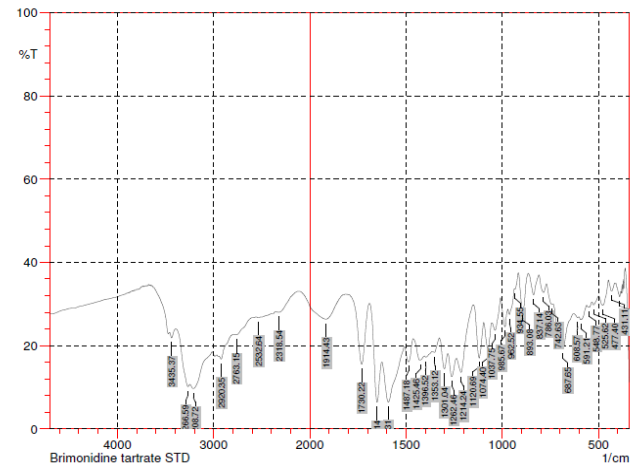


	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	390.6	31.7157	1.5075	404.1	385.78	8.9099	0.1986
2	431.11	33.1314	2.2197	448.47	416.64	14.8803	0.5155
3	477.4	29.7246	3.8552	498.62	449.43	24.7509	1.5966
4	525.62	29.7855	1.0691	534.3	499.58	17.8298	0.2788
5	548.77	29.2336	0.8463	561.31	535.27	13.7687	0.1932
6	591.21	26.255	1.4123	601.82	562.27	22.2505	0.5808
7	608.57	26.7598	0.3428	628.82	602.78	14.7438	0.0644
8	687.65	19.2178	0.3379	689.58	629.79	37.0816	0.1366
9	742.63	29.9448	0.7901	770.6	737.8	16.4445	0.344
10	786.03	32.7954	2.8594	809.17	771.56	17.6004	0.8457
11	837.14	32.2167	4.9468	864.15	810.14	24.752	1.532
12	893.08	28.2883	9.1353	916.23	865.11	24.8092	2.9855
13	934.55	33.5708	0.6407	937.44	917.19	9.2337	0.1558
14	962.52	27.4853	2.4174	970.24	938.41	16.9064	0.782
15	985.67	24.5733	5.2283	1008.81	971.2	21.2091	1.5701
16	1037.75	23.7128	3.9683	1054.14	1009.78	25.9285	1.6586
17	1074.4	19.2768	6.2303	1096.58	1055.11	27.2114	2.607
18	1120.69	17.0394	10.4086	1149.62	1097.54	34.1068	4.9616
19	1214.24	13.5853	6.8222	1237.39	1150.59	60.2268	4.4277
20	1262.46	12.5864	4.9912	1282.72	1238.35	36.7119	3.1398
21	1301.04	14.4567	5.1351	1327.08	1283.68	33.1727	2.6877
22	1353.12	18.3636	1.1746	1363.73	1328.05	25.2981	0.4985
23	1396.52	17.2501	0.4633	1404.24	1364.7	29.6409	0.2341
24	1425.46	16.581	0.0473	1426.42	1405.2	16.3222	0.033
25	1487.18	16.1449	4.4741	1507.43	1464.03	32.2439	2.5416
26	1592.31	6.4507	10.6261	1625.1	1508.4	107.7679	20.2969
27	1651.14	6.3946	15.2334	1689.72	1626.06	53.4909	11.6415
28	1730.22	15.5367	15.4306	1793.88	1690.68	63.3645	11.1323
29	1914.43	26.3817	0.251	1918.29	1823.77	50.5801	0.3195
30	2318.54	28.0338	0.2446	2326.25	2122.75	104.734	0.3924
31	2532.64	26.7805	0.0826	2557.72	2511.43	26.4572	0.0314
32	2763.15	22.6077	0.5744	2788.22	2558.68	139.0945	0.6988
33	2920.35	16.7913	1.3715	2940.61	2789.19	105.6683	0.8108
34	3208.72	9.7153	2.2284	3245.37	3015.83	199.5979	6.0928
35	3266.59	10.1638	2.3997	3391	3246.34	116.1657	3.3022
36	3435.37	21.8959	1.5796	3456.59	3391.97	41.3123	0.8201

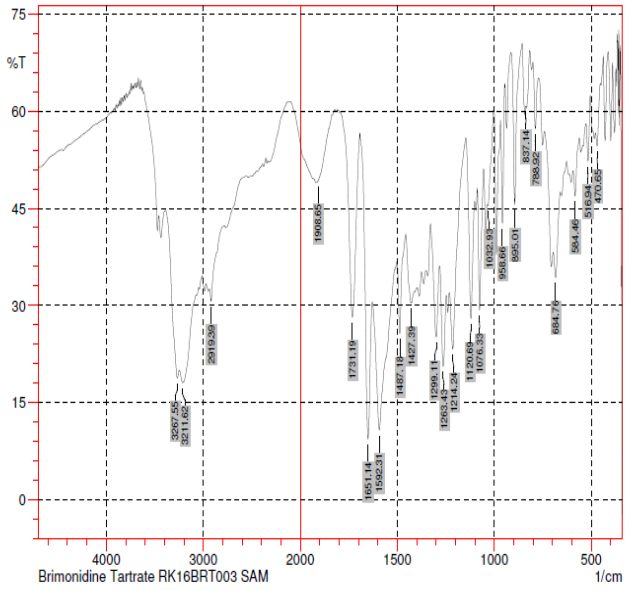
Date/Time:08/02/2016

Sample Name: Brimonidine tartrate Standard.spc

Apodization;

Resolution;

Figure 3: Dorzolamide Hydrochloride Standard spectrum



	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	470.65	54.6087	4.5678	479.33	453.29	6.101	0.3869
2	516.94	52.2642	7.9101	527.55	505.37	5.528	0.6436
3	584.46	46.868	5.4705	594.1	562.27	9.2568	0.5511
4	684.76	34.2924	6.9195	696.33	660.65	14.5417	1.3141
5	788.92	57.3409	9.2109	801.46	772.52	5.9422	0.7921
6	837.14	60.7273	0.0205	838.11	836.18	0.4177	0.0001
7	895.01	45.5807	23.9663	914.3	856.43	12.1518	3.1067
8	958.66	42.64	18.4013	968.31	946.12	6.2641	1.5568
9	1032.93	45.416	1.4499	1035.82	1002.06	9.5974	0.161
10	1076.33	29.2682	19.8213	1086.93	1055.11	12.86	3.1725
11	1120.69	27.9613	22.304	1146.73	1101.4	18.1083	4.7207
12	1214.24	23.2413	13.6655	1231.6	1147.69	34.6798	3.9027
13	1263.43	20.5508	13.0774	1281.75	1248	19.4619	3.5088
14	1299.11	25.1078	13.2729	1329.01	1282.72	22.7788	3.9142
15	1427.39	30.3581	0.1984	1428.35	1410.99	8.7484	-0.01
16	1487.18	25.626	11.4525	1500.68	1473.68	13.6198	1.9877
17	1592.31	10.7201	21.3692	1627.99	1514.19	75.5144	20.6704
18	1651.14	9.3708	30.4538	1688.75	1628.95	35.175	12.1262
19	1731.19	28.1916	29.6775	1793.88	1693.57	34.8727	11.3271
20	1908.65	49.2362	0.1508	1909.61	1852.71	15.8288	0.2145
21	2919.39	30.6384	3.1437	2936.75	2800.76	58.6278	0.774
22	3211.62	18.0812	4.205	3245.37	3074.66	109.1889	7.2132
23	3267.55	18.7488	4.9135	3389.08	3246.34	76.5048	3.5263

Date/Time:08/02/2016

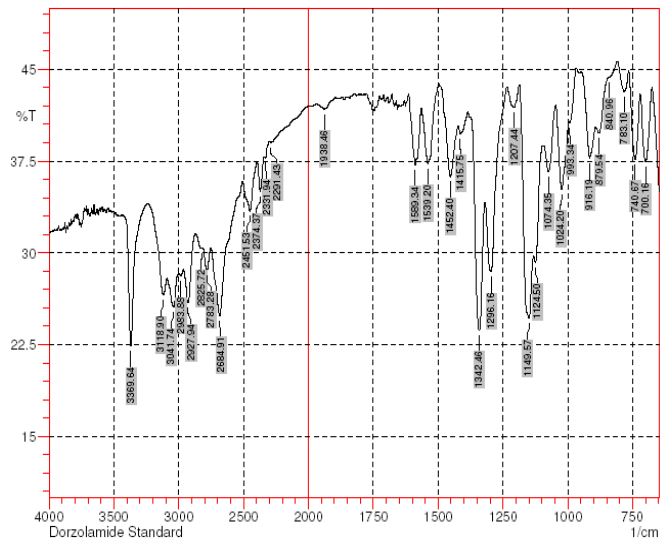
Sample Name: Brimonidine tartrate sample RK12BRT002.spc

Apodization;

Resolution;

Figure 4: Timolol Maleate sample IR spectrum

ISSN: 0975-8585



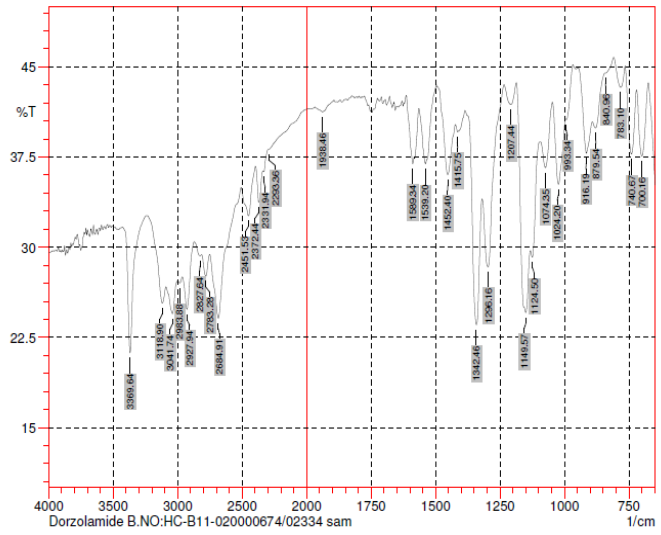
	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	700.16	37.435	5.684	719.45	677.01	17.038	1.551
2	740.67	37.648	6.153	763.81	721.38	16.824	1.624
3	783.1	43.181	2.042	808.17	765.74	15.129	0.522
4	840.96	44.353	0.089	842.89	810.1	11.419	0.063
5	879.54	39.783	1.508	891.11	844.82	17.568	0.316
6	916.19	37.726	4.485	947.05	893.04	21.285	1.165
7	993.34	40.6	0.387	995.27	968.27	10.039	0.141
8	1024.2	35.219	6.195	1045.42	997.2	20.357	1.841
9	1074.35	36.614	3.652	1097.5	1047.35	20.723	0.996
10	1124.5	29.228	2.536	1132.21	1097.5	16.503	0.471
11	1149.57	24.643	9.217	1186.22	1134.14	27.529	4.294
12	1207.44	41.908	1.725	1234.44	1188.15	17.173	0.496
13	1296.16	28.477	8.283	1319.31	1236.37	37.244	3.007
14	1342.46	23.683	12.854	1384.89	1321.24	31.712	4.706
15	1415.75	39.765	0.736	1421.54	1386.82	13.685	0.221
16	1452.4	36.219	5.532	1490.97	1423.47	27.407	1.906
17	1539.2	37.274	5.287	1564.27	1498.69	25.987	1.821
18	1589.34	37.194	1.513	1614.42	1583.56	12.443	0.246
19	1938.46	41.739	0.671	1967.39	1894.1	27.501	0.246
20	2291.43	38.997	0.146	2299.15	2017.54	110.82	0.193
21	2331.94	37.776	0.312	2339.65	2301.08	16.01	0.041
22	2374.37	34.991	2.633	2395.59	2341.58	23.725	0.825
23	2451.53	33.422	3.266	2507.46	2397.52	50.048	2.159
24	2684.91	24.881	7.028	2752.42	2509.39	125.853	8.948
25	2783.28	28.574	1.819	2814.14	2754.35	31.759	0.833
26	2825.72	30.361	0.258	2872.01	2816.07	28.595	0.136
27	2927.94	25.913	3.869	2962.66	2873.94	48.533	2.271
28	2983.88	28.122	0.316	2997.38	2964.59	17.974	0.087
29	3041.74	25.553	2.566	3091.89	2999.31	52.714	1.682
30	3118.9	26.53	2.439	3234.62	3093.82	73.215	1.473
31	3369.64	22.388	10.972	3429.43	3236.55	99.279	7.766

Date/Time:08/02/2016

Sample Name: Dorzolamide Standard.spc

Apodization;

Figure 5: Brimonidine tartrate sample spectrum



	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	700.16	37.584	5.693	719.45	677.01	16.966	1.548
2	740.67	37.757	6.189	763.81	721.38	16.766	1.627
3	783.1	43.308	2.064	808.17	765.74	15.069	0.524
4	840.96	44.484	0.089	842.89	810.1	11.379	0.066
5	879.54	39.915	1.514	891.11	844.82	17.511	0.321
6	916.19	37.839	4.517	947.05	893.04	21.209	1.169
7	993.34	40.605	0.388	995.27	968.27	10.024	0.14
8	1024.2	35.223	6.196	1045.42	997.2	20.359	1.845
9	1074.35	36.622	3.515	1093.64	1047.35	19.128	0.931
10	1124.5	29.174	2.452	1132.21	1095.57	17.308	0.44
11	1149.57	24.566	9.254	1186.22	1134.14	27.569	4.31
12	1207.44	41.871	1.735	1234.44	1188.15	17.189	0.498
13	1296.16	28.366	8.304	1319.31	1236.37	37.336	3.028
14	1342.46	23.569	13.207	1373.32	1321.24	27.335	4.933
15	1415.75	39.607	0.739	1421.54	1386.82	13.742	0.223
16	1452.4	36.043	5.505	1490.97	1423.47	27.544	1.899
17	1539.2	36.937	5.334	1564.27	1498.69	26.224	1.863
18	1589.34	36.954	1.506	1614.42	1583.56	12.532	0.247
19	1938.46	41.238	0.66	1967.39	1894.1	27.889	0.249
20	2293.36	38.132	0.055	2297.22	2017.54	112.049	0.107
21	2331.94	36.302	0.304	2337.72	2299.15	16.541	0.043
22	2372.44	33.742	2.816	2395.59	2339.65	25.418	0.946
23	2451.53	32.607	3.253	2507.46	2397.52	51.192	2.21
24	2684.91	24.096	6.808	2750.49	2509.39	127.912	8.859
25	2783.28	27.588	1.802	2814.14	2752.42	33.682	0.858
26	2827.64	29.304	0.294	2872.01	2816.07	29.469	0.142
27	2927.94	24.833	3.83	2962.66	2873.94	50.075	2.347
28	2983.88	27.02	0.308	2997.38	2964.59	18.541	0.089
29	3041.74	24.471	2.484	3089.96	2999.31	53.307	1.674
30	3118.9	25.347	2.465	3236.55	3091.89	77.926	1.587
31	3369.64	21.22	10.743	3427.51	3238.48	101.13	7.974

Date/Time:08/02/2016

Sample Name: Dorzolamide B.NO:HC-B11-020000674/02334.sample.spc

Figure 6: Dorzolamide Hydrochloride sample spectrum

IDENTIFICATION TEST RESULTS BY HPLC METHOD

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

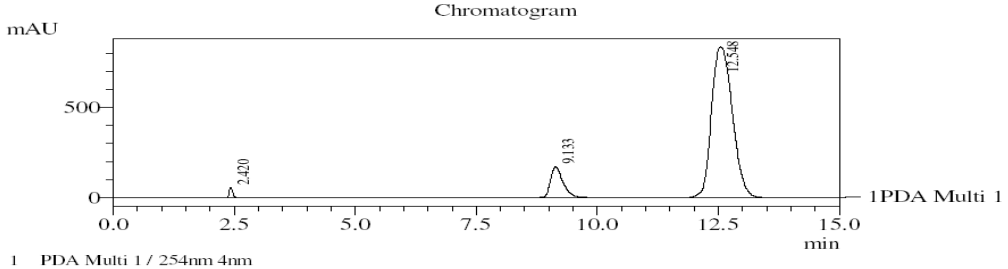
An HPLC study was carried out to check the identity of retention time for principal peak obtained with sample solution should corresponds with peak obtained with reference solution. The peak obtained from HPLC studies at wavelength of 254nm.

ISSN: 0975-8585

Sample Information

STD 2Acquired by	: Admin
Sample Name	: Timolol, Brimonidine, Dorzolamide
Sample ID	: BTD Standard
Injection Volume	: 20 uL
Data Filename	: Timolol, Bromonidine, Dorzolamide
Method Filename	: BTD drug analysis
Date Acquired	: 08.02.2016
Data Processed	: 08.02.2016

Chromatogram



1 PDA Multi 1 / 254nm 4nm

PeakTable

PDA Ch1 254nm 4nm

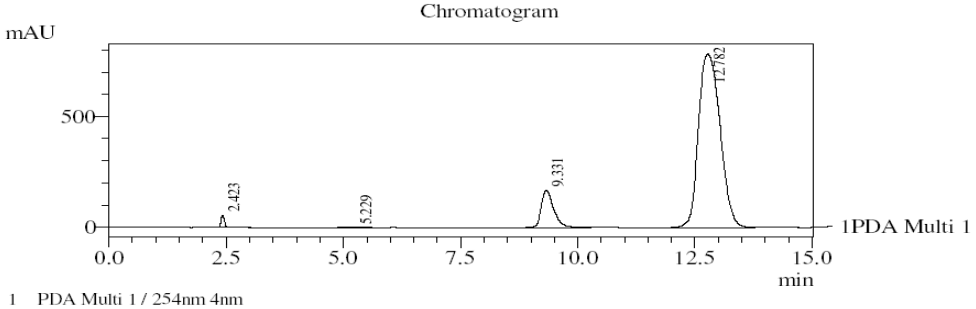
Peak#	Ret. Time	Area	Height	Height %	Theoretical Plate#
1	Timolol 2.420	271073	56050	5.270	4458.825
2	Brimonidine9.33	3160811	171447	16.119	5989.513
3	Dorzolamide12.548	25087656	836144	78.611	4228.743
Total		28519541	1063641	100.000	

Resolution	Tailing Factor
0.000	1.330
21.760	1.468
5.491	1.226

Sample Information

STD 3Acquired by	: Admin
Sample Name	: Timolol, Brimonidine, Dorzolamide
Sample ID	: BTD Sample
Injection Volume	: 20 uL
Data Filename	: Timolol, Bromonidine, Dorzolamide
Method Filename	: BTD drug analysis
Date Acquired	: 08.02.2016
Data Processed	: 08.02.2016

Chromatogram



1 PDA Multi 1 / 254nm 4nm

PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Height %	Theoretical Plate#
1	Timolol 2.423	271162	56263	5.578	4604.913
2	Brimonidine 9.331	3151445	168701	16.727	6040.268
3	Dorzolamide12.782	25102879	782989	77.634	3897.276
Total		28542741	1008567	100.000	

Resolution	Tailing Factor
0.000	1.335
6.167	1.091
6.576	1.478
5.313	1.228

ISSN: 0975-8585

Assay of Timolol Maleate by Non-aqueous titration: Limit: 98.5% - 101%
Titre value for Timolol Maleate= 8.1ml
Strength of 0.1M Perchloric acid= 1.0012
Weight of Timolol Maleate = 0.3501gm
Assay of Timolol Maleate

$$= \frac{\text{Titre value x strength of 0.1M Perchloric acid x Factor x 100}}{\text{Weight of substance}}$$

$$\text{Result} = \frac{8.1 \times 1.0012 \times 0.04325 \times 100}{0.3501} = \mathbf{100.18\%}$$

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 = 0.3508gm
Assay of Timolol Maleate

$$= \frac{\text{Titre value x strength of 0.1M Perchloric acid x Factor x 100}}{\text{Weight of substance}}$$

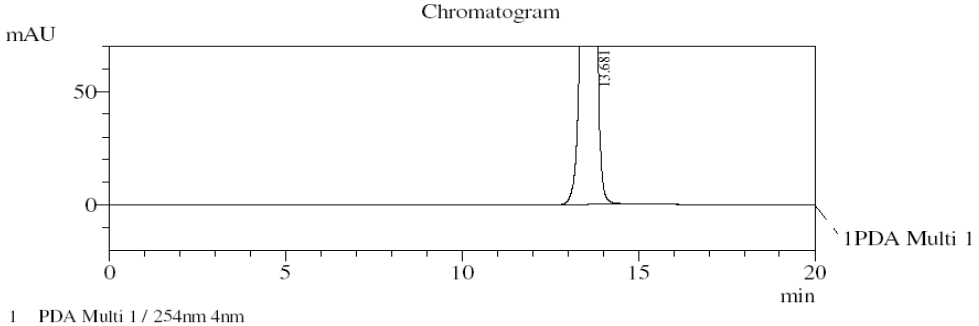
$$\text{Result} = \frac{7.9 \times 1.0012 \times 0.04422 \times 100}{0.3508} = \mathbf{99.70\%}$$

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

Sample Information

STD 2Acquired by	: Admin
Sample Name	: Dorzolamide standard
Sample ID	: Standard
Tray#	: 1
Vail#	: 14
Injection Volume	: 10 uL
Data Filename	: Dorzolamide Standard
Method Filename	: Dorzolamide RM USP.lcm
Batch Filename	: 08.02.2016
Report Filename	: Default.lcr
Date Acquired	: 08.02.2016
Data Processed	: 08.02.2016

Chromatogram



1 PDA Multi 1 / 254nm 4nm

PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Height %	Theoretical Plate#
1	Dorzolamide13.681	8814500	416552	100.000	9175.870
Total		8814500	416552	100.000	

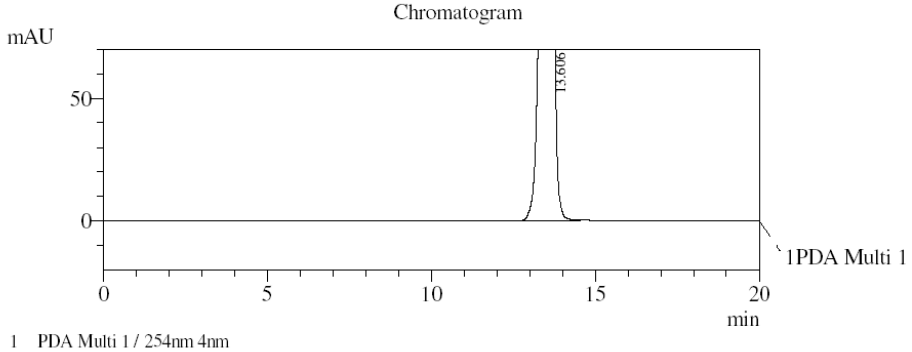
Resolution	Tailing Factor
0.000	0.782

ISSN: 0975-8585

Sample Information

SAM 1Acquired by	: Admin
Sample Name	: Dorzolamide sample
Sample ID	: HC B11 02 000674 02334
Tray#	: 1
Vail#	: 15
Injection Volume	: 10 uL
Data Filename	: Dorzolamide Standard
Method Filename	: Dorzolamide RM USP.lcm
Batch Filename	: 08.02.2016
Report Filename	: Default.lcr
Date Acquired	: 08.02.2016
Data Processed	: 08.02.2016

Chromatogram



1 PDA Multi 1 / 254nm 4nm

PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Height %	Theoretical Plate#
1	Dorzolamide13.606	8927285	416890	100.000	8761.381
Total		8927285	416890	100.000	

Resolution	Tailing Factor
0.000	0.772

$$= \frac{\text{Peak area sample x wt.of.std x 2 x 50 x 10 x 100}}{\text{Peak area of standard x 50 x 10 x wt.of.sample x 2}}$$

$$= \frac{8927285 \times 0.2212 \times 2 \times 50 \times 10 \times 100}{8814500 \times 50 \times 10 \times 0.2224 \times 2} = \mathbf{100.73\%}$$

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 has been consider before development of 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-formualtion 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.

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