



METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUS ESTIMATION OF TIMOLOL MALEATE AND TRAVOPROST IN BULK AND IN PHARMACEUTICAL DOSAGE FORM BY UV-SPECTROSCOPY

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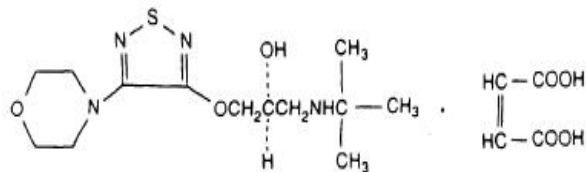
ABSTRACT

The combination of these two drugs (Timolol maleate and Travoprost) is used to lower the pressure in the eye when one of the medicines does not lower the pressure enough on its own. The eye drops should be layed into the affected eye(s) once per day. The aim of the present study is to develop new analytical method for the simultaneous estimation of Timolol maleate and Travoprost in bulk and in Pharmaceutical dosage form. Spectroscopic method have been developed for the quantification of Timolol maleate and Travoprost in bulk and in the formulation. In simultaneous equation method the absorbance of the Timolol maleate and Travoprost measured at 234nm and 280nm respectively by using double distilled water as blank which was further applied for determining the concentration of the both the drugs in formulation. The proposed method validated statistically for specificity, linearity, accuracy and precision. The LOD value of Timolol maleate and Travoprost was found to be 0.043, 0.0021 respectively. LOQ value of Timolol maleate and Travoprost was found to be 0.142, 0.0069 respectively. This method is validated as per ICH guidelines. This method is simple, cost effective, accurate and precise.

Keywords: Timolol maleate, Travoprost, Double distilled water, UV method.

INTRODUCTION

Drug Profile: Timolol Maleate



Chemical structure:

Systematic (IUPAC) name: 2-Propanol, 1- (1, 1-dimethylethyl) amino-3- [[4-(4-morpholinyl)-1, 2, 5-thiadiazol-3-yl] oxy]-, (S)-, (Z)-2-butenedioate (1:1) (salt). Blocks both β -1 and β -2 adrenergic receptors, reduces intraocular pressure by reducing aqueous humour production or possibly outflow; reduces blood pressure by blocking adrenergic receptors and decreasing sympathetic outflow, produces a negative chronotropic and inotropic activity through an unknown mechanism.

TRAVOPROST

Chemical structure

Systematic (IUPAC) name: [1R-[1 α (Z),2 β (1E,3R*),3 α ,5 α]]-7-[3,5-Dihydroxy-2-[3-hydroxy-4-[3-(trifluoromethylphenoxy)-1-butenyl] cyclopentyl

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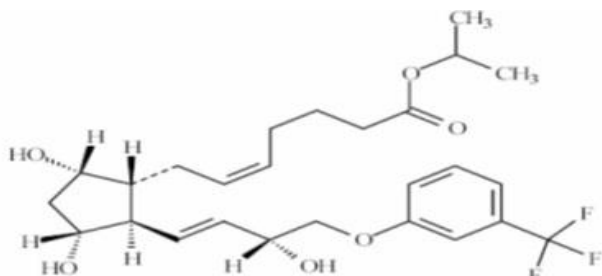


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]5-heptenoic acid, 1-methylethylester. Travoprost free acid is a selective FP prostanoid receptor agonist which is believed to reduce intraocular pressure by increasing trabecular meshwork and uveoscleral outflow. Travoprost is a type of medicine called a prostaglandin analogue. It helps in reduce the pressure in the eye by mimicking the action of a naturally-occurring prostaglandin. Prostaglandins are a group of natural body chemicals found in many places in the body. In the eye, they increase the drainage of the aqueous humour out of the eyeball. Travoprost is a synthetic prostaglandin that acts on the same receptors in the eye as the natural prostaglandins. It therefore causes an increase in the drainage of aqueous humour out of the eyeball. This decreases the pressure within the eye. Timolol is a type of medicine called a beta-blocker. These medicines block beta-receptors in various parts of the body. Blocking the beta receptors in the eye reduces the amount of aqueous humour that is produced. Timolol therefore reduces the inflow of aqueous humour into the eyeball and so decreases the pressure within the eye in a different way. The combination of these two medicines is used to lower the pressure in the eye when one of the medicines does not lower the pressure enough on its own. The eye drops should be put into the affected eye(s) once a day.



Till date only few analytical methods has been reported for the determination of Timolol maleate with other drugs combinations in ophthalmic solution by UV spectroscopy and RP-HPLC. There is no analytical method has been reported on Travoprost alone or any combination. The main aim in this work is to develop a new simple, sensitive, and cost efficient U.V Spectroscopic method for Timolol maleate and Travoprost for normal estimation of this drug present in the marketed dosage forms and to reduce the time of analysis based on the literature survey made. The main objective of the present study is to validate the developed method of Timolol maleate and Travoprost by UV spectroscopy with different parameters like Specificity, Linearity, Precision, Accuracy, Robustness, Limit of detection, and Limit of Quantification and Analytical technique for the estimation of the drug. Therefore, the present study is to develop a new, simple, rapid, efficient, reproducible and selective UV Spectroscopic method for the determination of Timolol maleate and Travoprost in bulk and in ophthalmic solution.

MATERIALS AND METHODS

Materials

Drug Sample (Raw material)

Travoprost and Timolol maleate were obtained from Lara Drugs Pvt. Limited., Nalgonda, India.

Formulation used

TravacomTM (Alcon laboratories (India) Pvt. Limited., Bangalore) – each ml containing 40 µg of Travoprost, Timolol maleate 6.8 mg equivalent to Timolol 5mg, was procured from Apollo pharmacy, Hyderabad.

Chemicals and solvents used

Double distilled water.

Instruments used

Different instruments used to carry out the present work,

- Balance (Sartorius- bsa224s-cw)
- UV-VIS 2060 double beam Spectrophotometer (Spectro plus)
- Micropipette
- Melting point apparatus
- FTIR- Bruker

METHOD

In the present work an attempt was made to develop and validate simple, precise and accurate method for the estimation of Travoprost and Timolol in pure form and in combined ophthalmic solution dosage form by UV Spectrophotometry.

UV Spectrophotometric Method

Selection of Solvent

The solubility of Travoprost and Timolol were determined in a variety of solvents as per Indian pharmacopoeia standards. Solubility test for Travoprost and Timolol were carried out in different polar and non-polar solvents. From the solubility studies double distilled water was selected as suitable solvent for proposed method.

Preparation of Standard Stock Solution

Preparation of standard Travoprost solution:

0.40mg of Travoprost raw material was accurately weighed and transferred into the 50 ml volumetric flask and dissolved in minimum quantity of double distilled water and made up to 50 ml with double distilled water. The solution was observed to contain 8µg/ml. Dilute 5 ml of this solution in to 25 ml with double distilled water. The solution was observed to contain 1.6µg/ml.

Preparation of standard Timolol solution:

6.8mg of Timolol raw material was accurately weighed and transferred into the 50 ml volumetric flask and dissolved in minimum quantity of double distilled water and made up to 50 ml with double distilled water. The solution was observed to contain 136 μ g/ml. Dilute 5 ml of this solution in to 25 ml with double distilled water. The solution was observed to contain 27.2 μ g/ml.

Selection of λ_{max}

The selection of wavelength for the estimation Travoprost and Timolol a suitable diluted stock solution contain 1.6 μ g/ml and 27.2 μ g/ml of each and the solutions were scanned between the ranges of 200-400nm using double distilled water as blank. After careful observation of the spectrums of the each drug solution of Travoprost and Timolol have the maximum absorbance at 280,234nm respectively. Stability was studied by measuring the same solution in different time intervals. It was observed that Travoprost and Timolol in double distilled water were stable for 1 hour.

Calibration graph

In this method, the aliquots of stock solution of Travoprost and Timolol (2.5 – 7.5 ml of 8 and 136 μ g/ml) were transferred into 25 ml volumetric flask and made up to the mark with double distilled water. The absorbance of different concentration solutions were measured at 280nm, 234nm against double distilled water as blank. The samples were found to be linear with the concentration range of 50 – 150%. The calibration curve was plotted using concentration against absorbance. The curves obtained were linear with the concentration range of 50 – 150%.

Estimation of Timolol maleate and Travoprost in ophthalmic solution

1ml solution was taken accurately (each ml containing 40 μ g of Travoprost, Timolol maleate 6.8 mg equivalent to Timolol 5mg) and transferred into the 50 ml volumetric flask and dissolved in minimum quantity of double distilled water and made up to 50 ml with double distilled water. From the clear solution, further dilutions were made by diluting 5 ml in to 25 ml volumetric flask and made up with double distilled water to obtain concentration of 1.6 μ g/ml Travoprost (27.2 μ g/ml of Timolol maleate) theoretically. The absorbance measurements were made 6 times for the formulation at 280nm, 234nm. From the absorptivity values of Travoprost and Timolol maleate at 280nm, 234nm and the amount could be determined by using simultaneous equation method.

Validation of developed method

Linearity

A calibration curve was plotted between concentration and absorbance. Travoprost and Timolol were linear with the concentration range of 50 – 150% at 280 nm, 234nm.

Accuracy (Recovery studies)

Accuracy

Three concentrations 50%, 100%, 150%, were injected in a triplicate manner and amount recovered and percentage recovery were calculated.

Sample preparation

50%: 0.3 ml solution was taken accurately (each ml containing 40 μ g (0.40 mg) of Travoprost, Timolol maleate 6.8 mg equivalent to Timolol 5mg) and add known standard of Travoprost and Timolol maleate (0.08 and 1.36 mg) transferred in to 50 ml of volumetric flask and added a minimum quantity of double distilled water with shake for 15 minutes and made up to the final volume with same double distilled water. Dilute 5 ml of this solution in to 25 ml with double distilled water.

100%: 0.3 ml solution was taken accurately (each ml containing 40 μ g (0.40 mg) of Travoprost, Timolol maleate 6.8 mg equivalent to Timolol 5mg) and add known standard of Travoprost and Timolol maleate (0.28 and 4.76 mg) transferred in to 50 ml of volumetric flask and added a minimum quantity of double distilled water with shake for 15 minutes and made up to the final volume with same double distilled water. Dilute 5 ml of this solution in to 25 ml with double distilled water.

150%: 0.3 ml solution was taken accurately (each ml containing 40 μ g (0.40 mg) of Travoprost, Timolol maleate 6.8 mg equivalent to Timolol 5mg) and add known standard of Travoprost and Timolol maleate (0.48 and 8.16 mg) transferred in to 50 ml of volumetric flask and added a minimum quantity of double distilled water with shake for 15 minutes and made up to the final volume with same double distilled water. Dilute 5 ml of this solution in to 25 ml with double distilled water.

Precision

1ml solution was taken accurately (each ml containing 40 μ g of Travoprost, Timolol maleate 6.8 mg equivalent to Timolol 5mg) and transferred in to 50 ml of volumetric flask and added a minimum quantity of double distilled water with shake for 15 minutes and made up to the final volume with same double distilled water. Dilute 5 ml of this solution in to 25 ml with double distilled water.

The repeatability of the method was confirmed by the analysis of formulation was repeated for three times with the same concentration. The amount of each drug present in the solution formulation was calculated. The percentage RSD was calculated. The intermediate precision of the method was confirmed by intraday and

inter day analysis i.e. the analysis of formulation was successive days. The amount of drugs were determined, percentage RSD also calculated.

LOD and LOQ

The linearity study was carried out for six times. The LOD and LOQ were calculated by using the average of slope and standard deviation of intercept.

RESULTS AND DISCUSSION

Estimation of multiple drug formulations have the advantage that the methods are time consuming and usage of solvent is minimized. Simple, rapid, precise, and accurate UV Spectrophotometric method was developed and validated for the estimation of Travoprost and Timolol in pure form and in combined solution dosage form.

UV SPECTROPHOTOMETRIC METHOD

The solubility of Travoprost and Timolol was determined as per Indian pharmacopoeia. Number of polar and non-polar solvents were tried to dissolve the drugs. From the solubility profile double distilled water was chosen as a common solvent for the estimation of Travoprost and Timolol. An FT-IR spectrum was recorded for Timolol maleate and Travoprost as shown in figure 1 and 2.

The sample solutions of 27.20µg/ml of Timolol maleate and 1.6µg/ml of Travoprost in double distilled water prepared individually and the solutions were scanned in UV region in the wavelength range from 200 to 400nm by using double distilled water as blank were shown in figures 3 and 4. From the spectrum 234nm for Timolol and 280 nm Travoprost, was selected as wavelength to construct simultaneous equation.

Different aliquots of Timolol and Travoprost in double distilled water were prepared in the concentrations percentage of 50-150%. The absorbances of solutions were measured at 234 and 280nm were shown in table 1-4 for Timolol maleate and Travoprost. The calibration curve was plotted using concentration percentage against absorbance. The calibration graph at 280 and 234nm for each drug was shown in figure 5-8 respectively. The preparation calibration curve was repeated for six times for each drug at their selective wavelengths. The optical

repeated three times in the same day and on three parameters like, sandell's sensitivity, molar absorptivities, correlation coefficient, slope, intercept, LOD, LOQ were calculated. The correlation coefficient for all the two drugs was found to be 0.999. This indicates that the two drugs obey Beer's law in the selected concentration range. Hence the concentrations were found to be linear. The optical characteristics of two drugs at their selective wavelength were shown in table 5-6 for estimation Timolol maleate and Travoprost.

The ophthalmic solution form (Travacom™ (Alcon laboratories (India) Pvt. Limited., Bangalore) – each ml containing 40 µg of Travoprost, Timolol maleate 6.8 mg equivalent to Timolol 5mg) was used for analysis. The concentration is 27µg/ml Timolol maleate and it is also containing 1.6µg/ml of Travoprost in double distilled water. The absorbances of the solution were measured at their wavelength. The percentage label claim present in ophthalmic solution was found to be 98.61 and 99.44% for Timolol maleate and Travoprost in 27µg/ml and 1.6µg/ml respectively. The amount present in ophthalmic solution was in good concord with the label claim and the % RSD values were found to be 0.2938901 and 0.3422546 for Timolol maleate and Travoprost in respectively. The low % RSD values indicate that the method has good precision. The results of analysis are shown in table 7.

Further the precision of the method was confirmed by intraday and inter day analysis. The analysis of ophthalmic solution was carried out for three times in the same day and one time in the three consecutive days. The % RSD value of intraday and inter day analysis was found to be 0.4002138 and 0.1768605 for Timolol maleate 27µg/ml and 0.4223977 and 0.3370725 for Travoprost 1.6µg/ml. The results of analysis are shown in table 8. The results shown that the precision of the method was confirmed.

The accuracy of the method was performed by recovery studies. The percentage recovery was found to be in the range of 100.07%, 99.84%, 99.84% for Timolol maleate, 99.78%, 99.91%, and 100.32% for Travoprost. The low % RSD value for two drugs indicates that this method is very accurate. The recovery data is shown in table 9.

Table 1. Average Absorbance of Timolol Maleate for Timolol Maleate at 234 nm

Sl.No	Con %	Abs-1	Abs-2	Abs-3	Abs-4	Abs-5	Abs-6	Avg. Abs
1	50	0.0922	0.0923	0.0925	0.0923	0.0922	0.0924	0.0922
2	75	0.1383	0.1385	0.1383	0.1384	0.1382	0.1385	0.1383
3	100	0.1832	0.1831	0.1835	0.1832	0.1834	0.1831	0.1832
4	125	0.2306	0.2305	0.2303	0.2306	0.2305	0.2304	0.2306
5	150	0.2767	0.2768	0.2767	0.2768	0.2765	0.2766	0.2767

Table 2. Average Absorbance of Timolol Maleate for Timolol Maleate at 280 nm

Sl.No	Con %	Abs-1	Abs-2	Abs-3	Abs-4	Abs-5	Abs-6	Avg. Abs
1	50	0.0955	0.0956	0.0954	0.0956	0.0957	0.0955	0.0955
2	75	0.1405	0.1405	0.1404	0.1406	0.1402	0.1406	0.1405
3	100	0.1845	0.1845	0.1843	0.1845	0.1842	0.1843	0.1842
4	125	0.2315	0.2315	0.2313	0.2314	0.2316	0.2314	0.2315
5	150	0.2774	0.2774	0.2772	0.2773	0.2771	0.2773	0.2771

Table 3. Average Absorbance of Travoprost for Travoprost at 234 nm

Sl.No	Con %	Abs-1	Abs-2	Abs-3	Abs-4	Abs-5	Abs-6	Avg. Abs
1	50	0.0268	0.0270	0.0269	0.0267	0.0268	0.0269	0.0269
2	75	0.0398	0.0399	0.0398	0.0399	0.0397	0.0398	0.0398
3	100	0.0509	0.0508	0.0509	0.0505	0.0508	0.0509	0.0509
4	125	0.0629	0.0629	0.0627	0.0628	0.0629	0.0627	0.0628
5	150	0.0742	0.0743	0.0745	0.0742	0.0742	0.0743	0.0742

Table 4. Average Absorbance of Travoprost for Travoprost at 280 nm

Sl.No	Con %	Abs-1	Abs-2	Abs-3	Abs-4	Abs-5	Abs-6	Avg. Abs
1	50	0.0246	0.0245	0.0246	0.0247	0.0244	0.0245	0.0245
2	75	0.0369	0.0368	0.0370	0.0368	0.0397	0.0367	0.0368
3	100	0.0482	0.0483	0.0484	0.0482	0.0483	0.0485	0.0483
4	125	0.0615	0.0614	0.0615	0.0613	0.0614	0.0612	0.0613
5	150	0.0738	0.0737	0.0738	0.0736	0.0738	0.0737	0.0737

Table 5. Optimal Characteristics of Timolol Maleate by UV Method

Parameters	At 234 nm	At 280 nm
Beers law limit	50-100%	50-100%
Sandwell's sensitivity ($\mu\text{g}/\text{cm}^2/0.001 \text{ A.U}$)	0.559947695	0.552079028
Molar absorptive ($\text{L mol}^{-1} \text{ cm}^{-1}$)	1022.924597	854.1970163
Correlation coefficient (r)	0.999137831	0.99916503
Slope(m)	0.032745881	0.032511336
Intercept(c)	0.00579317	0.001835724
LOD ($\mu\text{g}/\text{ml}$)	0.008215725	0.017722342
LOQ ($\mu\text{g}/\text{ml}$)	0.024896136	0.053704065
Standard error	2.92489	2.65328

Table 6. Optimal Characteristics of Travoprost by UV Method

Parameters	At 234 nm	At 280 nm
Beers law limit	50-100%	50-100%
Sandwell's sensitivity ($\mu\text{g}/\text{cm}^2/0.001 \text{ A.U}$)	2.157457168	2.069993926
Molar absorptive ($\text{L mol}^{-1} \text{ cm}^{-1}$)	442.1521386	255.348347
Correlation coefficient (r)	0.999002433	0.999112266
Slope(m)	0.008950176	0.008649765
Intercept(c)	0.00419823	0.000270347
LOD ($\mu\text{g}/\text{ml}$)	0.043245133	0.066891415
LOQ ($\mu\text{g}/\text{ml}$)	0.131045859	0.202701259
Standard error	2.48527	1.12931

Table 7. Estimation of Timolol Maleate and Travoprost in Ophthalmic Solution (Travacom_{TM}) by UV Method

S.No	Sample Weight(ml)	Timolol Maleate(abs)	Travoprost (abs)	%Assay (TM)	%Assay (TRA)
1	1	0.1845	0.0492	98.56	99.05
2	1	0.1840	0.0491	99.15	99.89
3	1	0.1842	0.0494	98.40	99.49
4	1	0.1842	0.0495	98.40	99.7
5	1	0.1848	0.0492	98.72	99.05
6	1	0.1843	0.0494	98.45	99.49
Average mean				98.61	99.44
STD				0.289805	0.340338
% RSD				0.2938901	0.3422546

Table 8. Intra Day and Inter Day Analysis of Ophthalmic Solution (Travacom_{TM}) - by UV Method

S.No	Sample Weight (ml)	Assay in %(Timolol maleate)		S.D*		% RSD*	
		Intra day	Inter day	Intra day	Inter day	Intra day	Inter day
1	1	98.56	98.40	0.395011	0.174243	0.4002138	0.1768605
2	1	99.15	99.72				
3	1	98.40	98.45				
Mean		98.70	98.52				

S.No	Sample Weight(ml)	Assay in %(Travoprost)		S.D*		% RSD*	
		Intra day	Inter day	Intra day	Inter day	Intra day	Inter day
1	1	99.05	99.7	0.420159	0.331713	0.4223977	0.3370725
2	1	99.89	99.05				
3	1	98.49	99.49				
Mean		99.47	98.41				

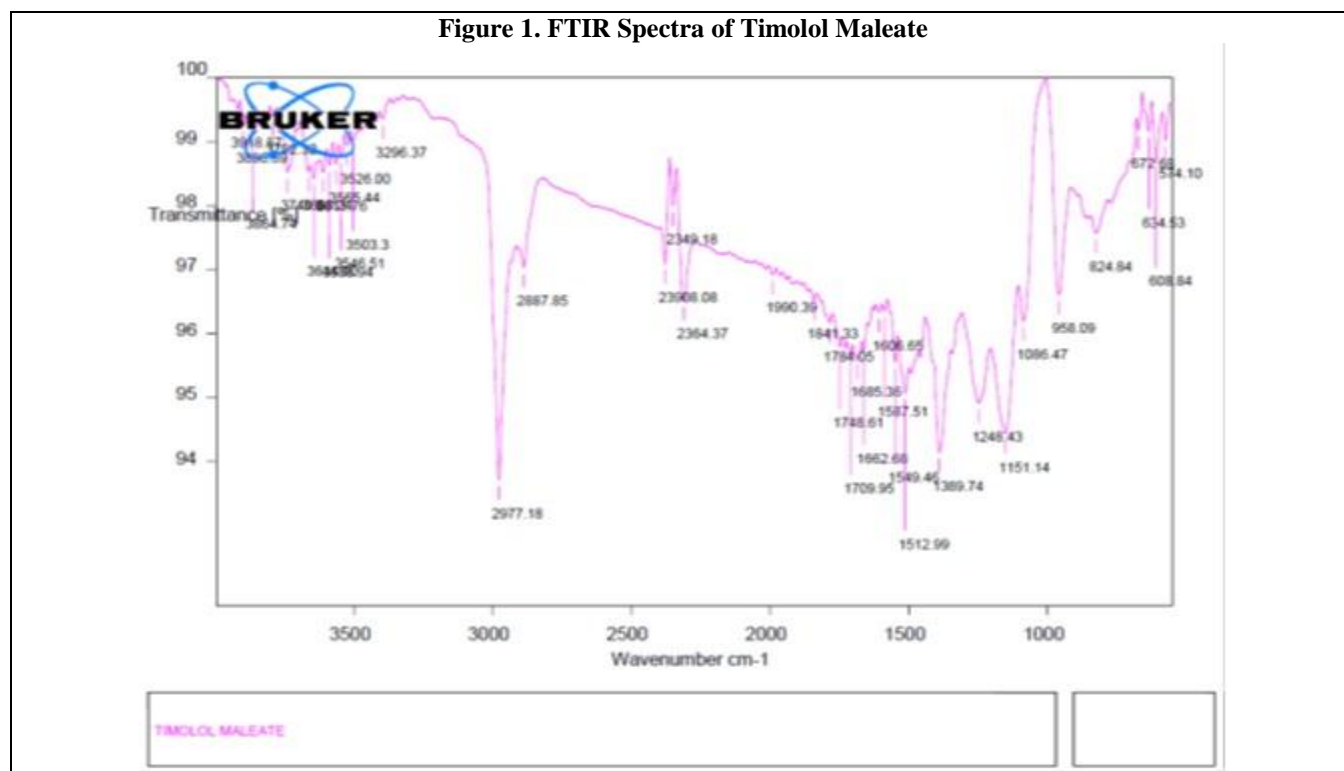
Table 9a. Recovery Analysis of Ophthalmic Solution-(Travacom_{TM}) - by UV Method

TIMOLOL MALEATE						
Spiked level	Sample weight (ml)+ spiking standard added	Sample absorbance	µg/ml added	µg/ml found	% Recovery	% Mean
50%	0.3+1.36	0.0922	335.274	334.942	99.90	100.07
50%	0.3+1.36	0.0924	335.274	335.668	100.11	
50%	0.3+1.36	0.0925	335.274	336.031	100.22	
100%	0.3+4.76	0.1843	670.548	669.521	99.84	99.84
100%	0.3+4.76	0.1845	670.548	670.247	99.95	
100%	0.3+4.76	0.1843	670.548	669.521	99.84	
150%	0.3+8.16	0.2767	1005.822	1005.189	99.93	99.84
150%	0.3+8.16	0.2766	1005.822	1002.646	99.68	
150%	0.3+8.16	0.2767	1005.822	1005.189	99.93	

Table 9b. Recovery Analysis of Ophthalmic Solution-(Travacom™) - by UV Method

TRAVOPROST						
Spiked level	Sample weight (ml)+ spiking standard added	Sample absorbance	µg/ml added	µg/ml found	% Recovery	% Mean
50%	0.3+0.08	0.0247	19.888	19.899	100.05	99.78
50%	0.3+0.08	0.0245	19.888	19.738	99.24	
50%	0.3+0.08	0.0247	19.888	19.899	100.05	
100%	0.3+0.28	0.0493	39.776	39.718	99.85	99.91
100%	0.3+0.28	0.0494	39.776	39.799	100.05	
100%	0.3+0.28	0.0493	39.776	39.718	99.85	
150%	0.3+0.48	0.0741	59.664	59.699	100.05	100.32
150%	0.3+0.48	0.0743	59.664	59.860	100.32	
150%	0.3+0.48	0.0745	59.664	60.021	100.59	

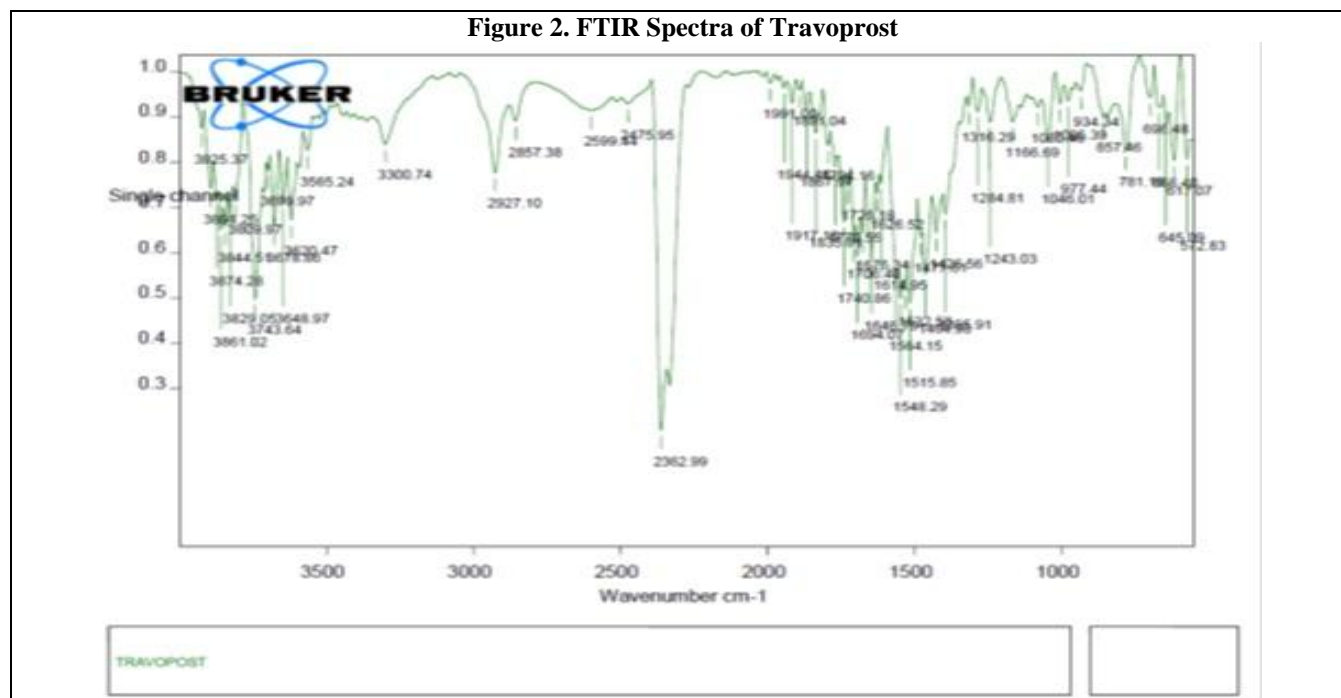
Figure 1. FTIR Spectra of Timolol Maleate



S. No	Frequency cm ⁻¹	Bond	Functional group
1	3564.44	=NOH(OH Stretch)	Misc
2	3526.00	O-H Stretching	Alcohols
3	3296.37	# C-H Stretch	Alkynes
4	3503.3	NH Stretch	Amides
5	3546.51	O-H Stretching	Alcohols
6	2977.18	CH Stretch	Alkanes
7	2887.85	CH Stretch	Alkanes
8	2349.18	Si-H Silane	Misc
9	2364.37	P-H Phosphine sharp	Misc
10	1990.39	N=C in R-N=C=S	Misc
11	1784.05	C-O Stretch	Ketones
12	1606.65	C-C Stretch	Alkenes
13	1685.36	Dimer C-O Stretch	Carboxylic Acid

14	1587.51	Ar C-C Stretch	Aromatics
15	1748.61	C-O Stretch doublet	Ketones
16	1662.68	C=C Stretch	Alkenes
17	1549.46	NH Out of plane	Amides
18	1709.95	Dimer C=O	Carboxylic Acid
19	1389.74	S=O Sulfate ester	Misc
20	1512.99	N=O Nitroso	Misc
21	1248.43	Si-CH3(Sharp)	Misc
22	1511.14	N=O Nitroso	Misc
23	1086.47	C-O Stretch	Ethers
24	958.09	=NOH (OH Stretch)	Misc
25	824.84	CH Out of plane	Aromatics
26	608.84	# C-H Bend	Alkynes
27	634.53	# C-H Bend	Alkynes
28	672.68	# C-H Bend	Alkynes
29	574.10	C-Br stretch	Alkyl halides

Figure 2. FTIR Spectra of Travoprost



S. No	Frequency cm^{-1}	Bond	Functional group
1	3565.24	=NOH(OH Stretch)	Misc
2	3599.27	=NOH(OH Stretch)	Misc
3	3620.47	O-H Free hydroxyl	Alkanes
4	3300.74	# C-H Stretch	Alkynes
5	2927.10	-CH2-	Alkanes
6	2857.38	CH Stretch	Alkanes
7	2599.44	S-H (sharp)	Misc
8	2362.99	P-H Phosphine sharp	Misc
9	1991.00	N=C in R-N=C=S	Misc
10	1626.52	N-H Out of plane	Amides
11	1726.18	C=O Stretch	Aldehydes
12	1676.34	C=C Stretch	Alkenes

13	1740.86	C=O Stretch	Esters
14	1584.15	NH2 In plane bend	Amines
15	1515.85	N=O Nitroso	Misc
16	1548.29	NH out of plane	Amide
17	1316.29	S=O sulfone I	Misc
18	1284.81	SI-CH3(sharp)	Misc
19	1243.03	SI-CH3(sharp)	Misc
20	1166.69	C-Br stretch	Alkyl halides
21	1046.01	C-O Stretch	Alcohols
22	977.44	N-O Alifatic	Misc
23	934.34	=NOH (N-O)	Misc
24	857.46	C-H Out of plane	Aromatics
25	696.48	C-H Out of plane	Aromatics
26	645.09	# C-H Bend	Alkynes
27	572.83	C-Br stretch	Alkyl halides

Figure 3. UV Spectrum of Timolol Maleate

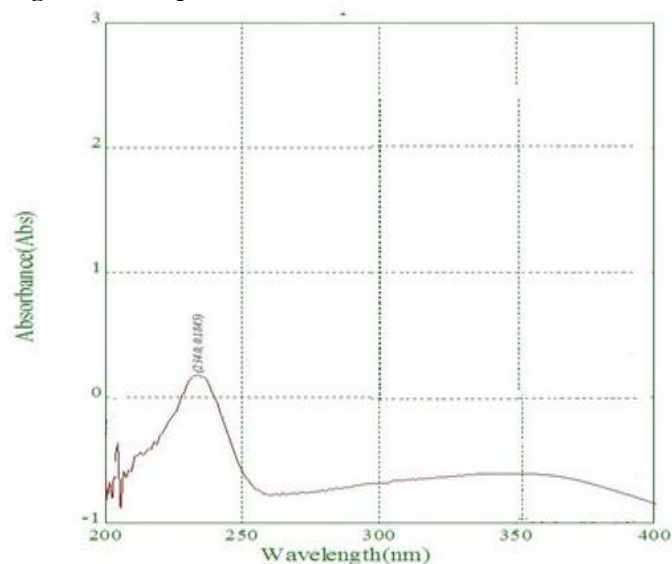


Figure 4. UV Spectrum of Travoprost

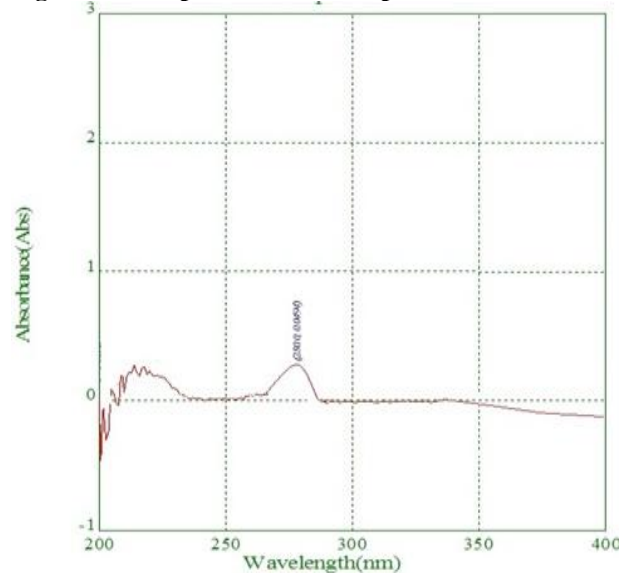


Figure 5. Calibration Curve of Timolol Maleate by UV-Spectroscopy at 234 nm

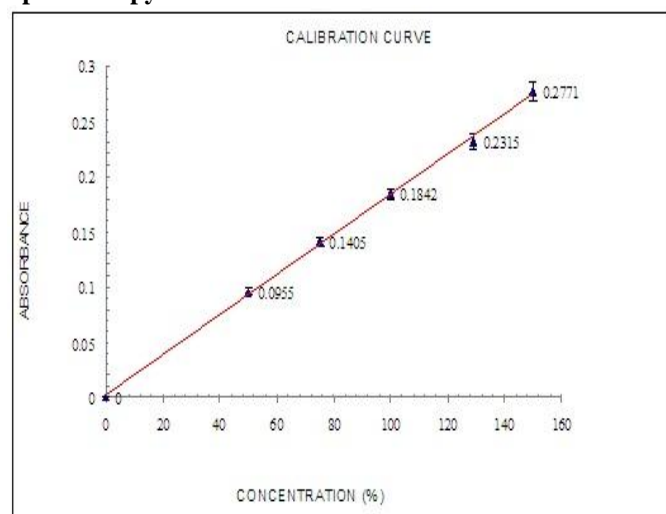


Figure 6. Calibration Curve of Timolol Maleate by UV- Spectroscopy at 280nm

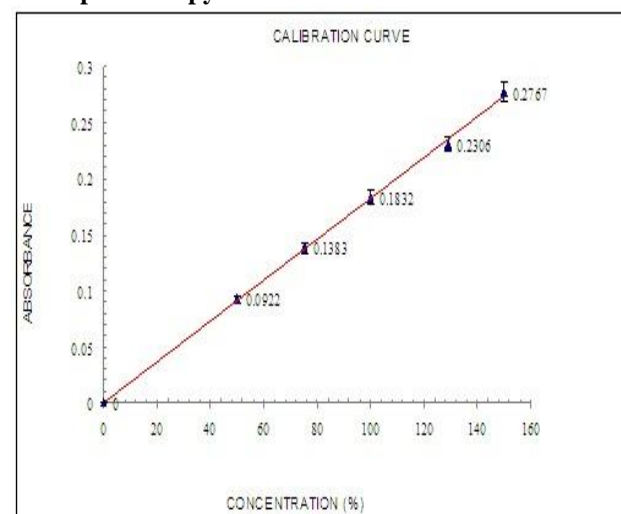


Figure 7. Calibration Curve of Travoprost by UV-Spectroscopy at 234nm

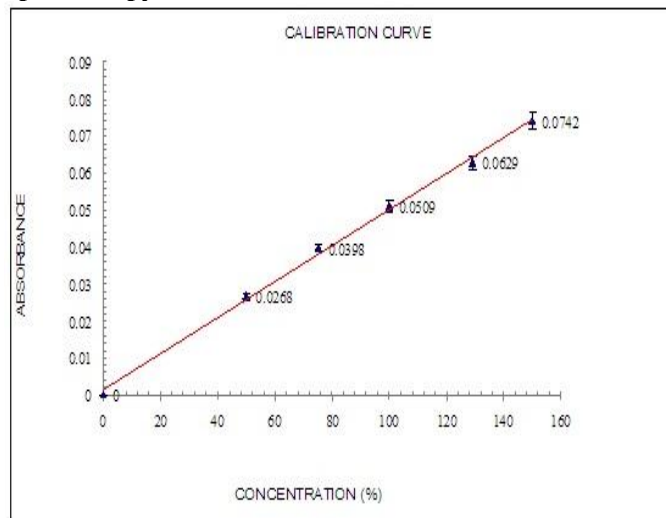
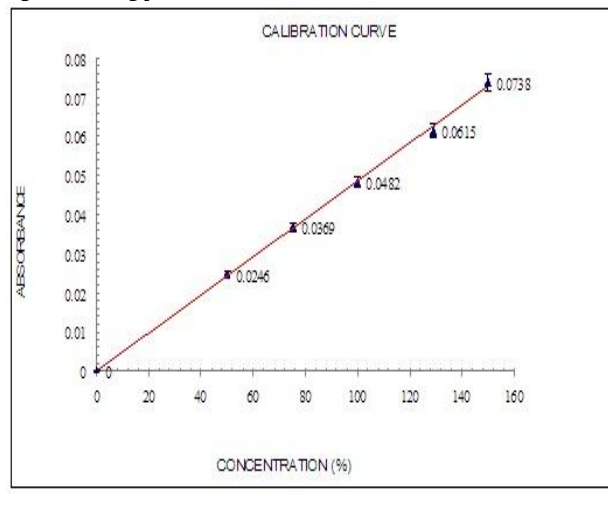


Figure 8. Calibration Curve of Travoprost by UV-Spectroscopy at 280nm



CONCLUSION

Simple, rapid and accurate analytical method was developed for the determination of Timolol maleate and Travoprost in bulk and in ophthalmic solution by using UV Spectrophotometry. The method was shown that excellent sensitivity, reproducibility, accuracy, repeatability, which is evidenced by the low percentage relative standard deviation. The results obtained from recovery studies were indicating that there was no interference with the excipients used in the ophthalmic solution. Hence it is suggested that the

proposed UV Spectrophotometric method may work effectively for routine analysis of Timolol maleate and Travoprost in bulk and in ophthalmic solution.

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