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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF BIMATOPROST AND TIMOLOL MALEATE

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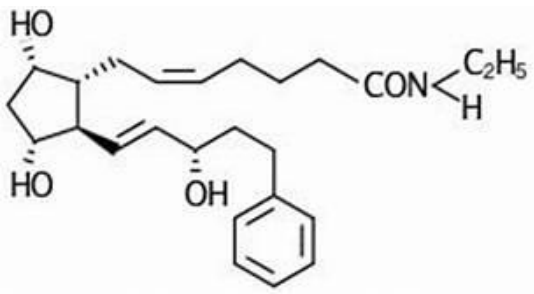
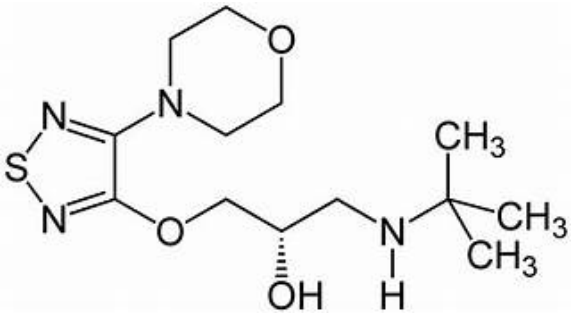
Abstract: Simple, specific and inexpensive RP-HPLC method has been developed for the estimation of Bimatoprost and timolol in bulk and pharmaceutical formulations. The mobile phase used was a mixture of Methanol and Water OPA 0.1%. The wavelength used for detection of Bimatoprost and timolol was 254 nm. The RP-HPLC method for respective linear equation for Bimatoprost was y = 136.3x+6.709 where x is the concentration and y is area of peak. The correlation coefficient was 0.999. The method was validated with respect to accuracy, precision, linearity and robustness as per the ICH Guidelines.

Introduction: Bimatoprost is a structural analog of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Like other $PGF_{2\alpha}$ analogs like travoprost, latanoprost and tafluprost, it shows increase in the outflow of aqueous fluid and lowers intraocular pressure in eye. However, in contrast to those it doesn't act on the prostaglandin F receptor, nor on any other known prostaglandin receptor. It is thought that bimatoprost mimics the human body's own prostamides (which are chemically similar), a category of drug associated with class prostaglandins, but with an unknown mechanism of action. Timolol competes with adrenergic neurotransmitters for binding to beta (1)-adrenergic receptors within the heart and therefore the beta (2)-receptors within the vascular and bronchial smooth muscle. This results in diminished actions of catecholamines, which normally bind to adrenergic receptors and exert sympathetic effects resulting in a rise in blood pressure and heart rate. Beta(1)-receptor blockade by timolol results in a decrease in both heart rate and cardiac output during rest and exercise, and a decrease in both systolic and diastolic blood pressure. In addition to the present, a reduction in reflex orthostatic hypotension may also occur.



(a) Bimatoprost



(b) Timolol

Materials and Methods: Bimatoprost and timolol was obtained from Analytical research lab Ahmedabad , Orthophosphoric acid(OPA) was purchased from Avantor Performance material India Ltd. Thane, Maharashtra (Merck), Methanol HPLC grade (Merk) and water of HPLC grade (Merck). Commercially available formulation of Bimatoprost and Timolol (Careprost plus) was procured from local market. The analysis of the drug was carried out on Younglin (S.K.) Gradient System UV Detector. Equipped with Reverse Phase (Cosmosil) C18 column (4.6mm x 250mm; 5µm), a SP930Dpump, a 20µl injection loop and UV730D Absorbance detector and running autochro-3000 software.

Preparation of Mobile phase:

Sr. No.	Mobile Phase
1.	Methanol+ Water OPA 0.1%(70:30 % v/v)0.7ml 254 nm
2.	Methanol+ Water OPA 0.1% (70:30 % v/v) 1 ml 254 nm
3	Methanol+ Water OPA 0.1% (80:20 % v/v) 1.3ml 254 nm
4	Methanol+ Water OPA 0.1% (60:40 % v/v) 0.8ml 254 nm
5	Methanol+ Water OPA 0.1% (60:40 % v/v) 0.9ml 254 nm
6	Methanol+ Water OPA 0.1% (45:55 % v/v) 1ml 254 nm

Preparation of standard stock solution:-

- **Preparation of std. Timolol solution: (Stock I)**

From the freshly prepared standard stock solution (1666ug/ml), 0.1 -0.5 ml stock solution was pipetted out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration of 16.66-83.30 ug/ml.

- **Preparation of std.Bimatoprost solution: (Stock II)**

From the freshly prepared standard stock solution (100ug/ml), 0.1-0.5 ml stock solution was pipetted out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 1-5 ug/ml.

- **Preparation of std. Timolol and Bimatoprost solution :(Stock III)**

From the freshly prepared standard stock solution (1666 ug/ml)Timolol,and (100 ug/ml)Bimatoprost , 0.1-0.5 ml stock solution was pipetted out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 16.66-83.30 ug/ml for Timolol and Bimatoprost 1-5 ug/ml respectively.

Evaluation of Analytical Methods:

Linearity:

The data obtained in the calibration experiments when subjected to linear regression analysis showed a linear relationship between peak areas and concentrations in the range 16.66-83.30 µg/mL for Timolol and 1-5 µg/mL for Bimatoprost (**Table No: 1, and Table No:2)** depict the calibration data of Timolol and Bimatoprost The respective linear equation for Timolol was y = 9.378x – 6.505 and Bimatoprost equation y = 136.3x + 6.709 where x is the concentration and y is area of peak. The correlation coefficient was 0.9987. The calibration curve of Timolol and Bimatoprost is depicted in (**FigNo.1 and Fig No.2)**

Table No 1: Linearity data for Timolol

Method	Conc µg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
RP-HPLC Method	16.66	144.25	146.26	145.26	1.42	0.98
	33.32	309.471	303.03	306.25	4.55	1.49
	49.98	478.61	475.63	477.12	2.11	0.44
	66.64	625.34	616.33	620.84	6.37	1.03
	83.30	772.04	761.28	766.66	7.61	0.99
	Equation		y = 9.378X+6.505			
	R^2		0.9987			

Table No 2: Linearity data for Bimatoprost

Method	Conc µg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
RP-HPLC Method	1	139.5941	138.8348	139.21	0.54	0.39
	2	285.6116	279.6803	282.65	4.19	1.48
	3	422.164	415.7184	418.94	4.56	1.09
	4	555.9138	547.0524	551.48	6.27	1.14
	5	688.0223	685.1	686.56	2.07	0.30
	Equation		y = 136.3x + 6.709			
	R^2		0.9991			

Accuracy: Accuracy of RP-HPLC method is ascertained by recovery studies performed at different levels of concentrations (80%, 100% and 120%). The % recovery was found to be within 99-101%.

Table 3. Result of Recovery data for Timolol and Bimatoprost

METHOD	Drug	Level (%)	Amt. taken (µg/ml	Amt. Added (µg/ml	area Mean* ± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean *± S.D.
RP-HPLC Method	TIMO	80%	16.66	13.32	29.99±0.12	13.33±0.12	100.08±0.90
		100%	16.66	16.66	33.05±0.002	16.39±0.002	98.40±0.01
		120%	16.66	19.99	36.76±0.14	20.10±0.14	100.54±0.70
	BIM	80%	1	0.8	1.80±0.002	0.80±0.002	100.59±0.19
		100%	1	1	2.01±0.014	1.01±0.014	100.71±1.42
		120%	1	1.2	2.21±0.008	1.21±0.008	100.55±0.67

***mean of each 3 reading for RP-HPLC method**

Table.4. Statistical Validation of Recovery Studies Timolol and Bimatoprost

METHOD	Level of Recovery (%)	Drug	Mean % Recovery	Standard Deviation*	% RSD
Rp-HPLC Method	80%	TIMO	100.08	0.90	0.90
		BIMA	100.59	0.19	0.19
	100%	TIMO	98.40	0.01	0.01
		BIMA	100.71	1.42	1.42
	120%	TIMO	99.81	0.70	0.70
		BIMA	100.55	0.67	0.67

***Denotes average of three determinations for RP-HPLC.**

Precision: Intra day and Inter day Precision studies on RP-HPLC for Timolol and Bimatoprost which shows the high precision %amount in between 97% to 100% indicates to analytical method that concluded.

Result of Intraday and Inter day Precision studies on RP-HPLC for Timolol and Bimatoprost

METHOD	Drug	Conc^{n} (µg/ml)	Intraday Precision		Interday Precision	
			Mean± SD	%Amt Found	Mean± SD	%Amt Found
Rp-HPLC METHOD	TIMO	33.32	306.31± 4.99	99.64	309.17±1.48	101.02
		49.98	475.35±6.34	100.9	475.62±0.70	102.86
		66.64	623.22±3.07	100.7	618.24±1.54	0.25
	BIMA	2	286.62± 2.98	8.174	286.75±0.57	102.73
		3	414.96±3.54	12.1	413.63±2.28	99.52
		4	546.79±2.97	16.14	549.99±0.94	99.65

***Mean of each 3 reading for RP-HPLC method**

Robustness: The mobile phase composition was changed in (±1 ml/min^{-1}**)** proportion and the flow rate was varied by(±1ml/min^{-1}**)**, and wavelength change(±1 ml/min^{-1}**)** of optimized chromatographic condition. The results of robustness studies are shown in **(Table No.5, 6**).Robustness parameters were also found satisfactory; hence the analytical method would be concluded.

Table No.5 Result of Robustness Study of Timolol

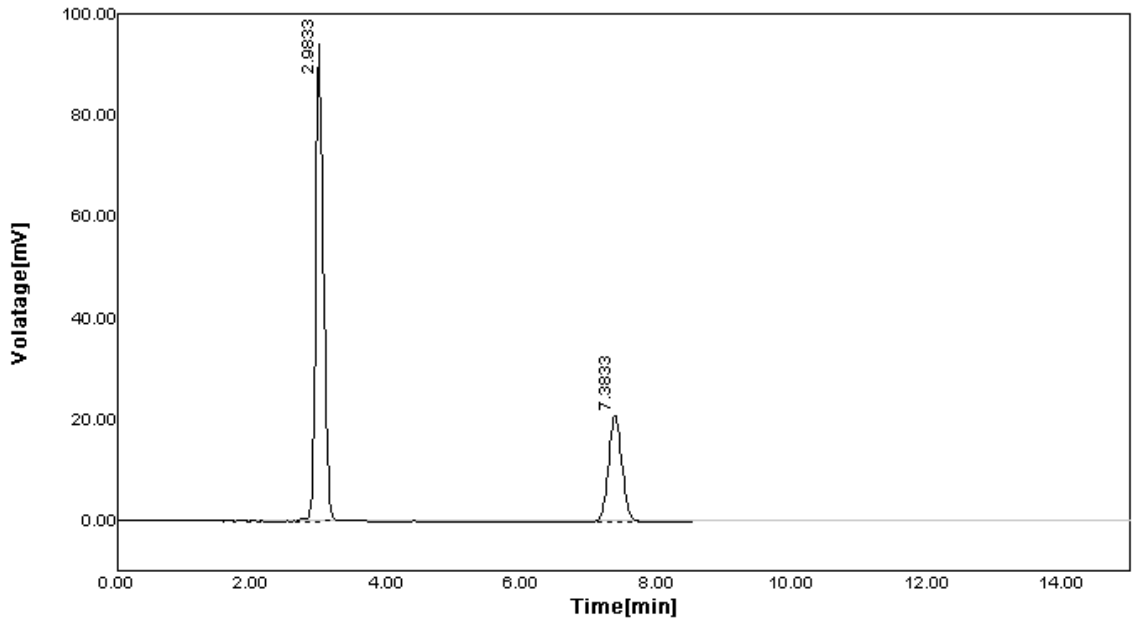
Parameters	Conc.(µg/ml)	Amount of detected(mean ±SD)	%RSD
Chromatogram of flow change 0.9ml	49.98	465.7±6.34	1.36
Chromatogram of flow change 1.1 ml	49.98	525.59±5.26	0.99
Chromatogram of comp change 46 MEOH +54 WATER	49.98	578.89±6.69	1.16
Chromatogram of comp change 44 Methanol+ 56 WATER	49.98	581.02±0.82	0.14
Chromatogram of comp change wavelength change 253nm	49.98	545.48±2.37	0.43
Chromatogram of comp change wavelength change 255nm	49.98	598.80±2.02	0.34

Table No.6. Result of Robustness Study of Bimatoprost

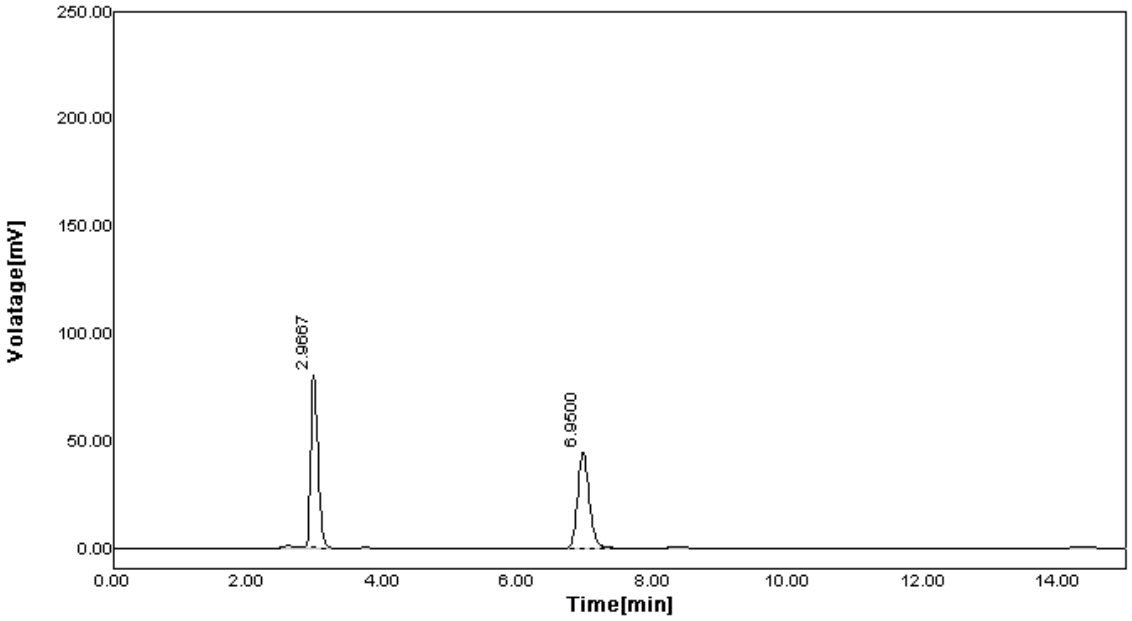
Parameters	Conc.(µg/ml)	Amount of detected(mean ±SD)	%RSD
Chromatogram of flow change 0.9ml	3	435.64±0.83	0.19
Chromatogram of flow change 1.1 ml	3	465.82±2.84	0.61
Chromatogram of comp change 46 METHANOL+54 WATER	3	468.36±7.01	1.50
Chromatogram of comp change 44METHANOL+ 56 WATER	3	479.37±8.55	1.78
Chromatogram of comp change wavelength change 253nm	3	486.20±8.39	1.72
Chromatogram of comp change wavelength change 255nm	3	475.96±7.69	1.61

Detection limit and quantitation limit: The LOD and LOQ of Timolol was found to be 4.6755(µg/mL) and 14.16(µg/mL), and The LOD and LOQ of Bimatoprost was found to be 0.7395(µg/mL) and 2.2412(µg/mL), analytical method that concluded.

Chromatogram of standard Combination of Timolol and Bimatoprost



Chromatogram for Marketed Formulation



Result and discussion: The present study was carried out to develop a sensitive, precise and accurate RP-HPLC method for the analysis of Bimatoprost and timolol maleate in pharmaceutical dosage form. The retention time for Bimatoprost and Timolol maleate was found to be 2.9 and 6.7 respectively. The results are presented in the form of %RSD which is below 1.00 and shows that the proposed HPLC method was highly precise. The method was robust as observed from insignificant variation within the results of study by changes in flow rate, Mobile phase composition and temperature. The drug content in the eye drop was quantified using the proposed analytical method. The proposed reversed phase HPLC method was found to be simple, precise, highly accurate, specific and no time consuming

Conclusion: The developed method was found to be simple, sensitive, accurate, precise and reproducible. It can be used for routine quality control analysis of Bimatoprost and Timolol maleate in ophthalmic solution

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