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RESEARCH ARTICLE

Stability Indicating RP-HPLC Method for Simultaneous Estimation Bimatoprost and Timolol Maleate in Bulk & Pharmaceutical Dosage Form

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ABSTRACT

In the method development for RP-HPLC method for Bimatoprost & Timolol Maleate in bulk drug dosage form with water: methanol as Diluents 10mints run time. Method was optimized by varying chromatographic parameters like column, mobile phase composition, mobile phase PH and flow rate to satisfy system suitability testing. Various columns and mobile phase combinations were tried. A satisfactory separation and good peak symmetry was obtained by using Intersil ODS (150×4.6mm, 5µ) column, Acetonitrile: 0.02M Potassium dihydrogen orthophosphate as mobile phase with gradient technique. Quantification was achieved with PDA detection at 213nm based on peak area. The assay results obtained by using the proposed method for the analysis of marketed ophthalmic solution containing Bimatoprost 4mg and Timolol maleate 5mg were in good agreement with the labelled amounts of Bimatoprost and Timolol maleate. The average contents of Bimatoprost and Timolol maleate were 3.8964 mg/ml (97.41 %) and 4.834 mg/ml (96.68%) respectively.

Keywords: Bimatoprost, Acetonitrile, Potassium dihydrogen orthophosphate

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CONTENTS

1. Introduction

Chromatographic conditions :
Flow rate : 1.0ml/min
Column : Intersil ODS, 150 x 4.6 mm, 5□.Detector
Wave length : 213nm
Column temperature: 25°C
Injection volume : 10□L
Run time: 10 min
Diluent : Water: Methanol (50:50) Mobile phase:
A gradient programme with mobile phase consisting of 0.02 M Potassium dihydrogen orthophosphate and acetonitrile was pumped at a flow rate of 1ml/min. A gradient programme was followed 1,2.

2. Materials and Methods

Marketed formulation:

Each 5ml ophthalmic solution (Duotrav) containing Bimatoprost (0.004%) and Timolol maleate (0.5%) was procured from local market.

Mobile phase preparation:

0.02M Potassium dihydrogen orthophosphate and Acetonitrile used for the mobile phase were filtered through 0.45µ membrane filter and degassed by ultrasonicator for 15min36, 37.

Preparation of Buffer: (0.02M KH2PO4)

Accurately weighed and transferred 2.72gm of Potassium dihydrogen Orthophosphate in a 1000ml of Volumetric flask add about 1000ml of Milli-Q water added add 1ml of Triethylamine and degassing to sonicate and finally make up the volume with water, then pH adjusted to 3.10 with dil. Ortho phosphoric acid solution19,20.

Preparation of standard stock solution:

Standard solution of Bimatoprost was prepared by dissolving 4mg of Bimatoprostin methanol: water (50:50) to get a solution containing 400µg/ml of Bimatoprost. Standard solution of Timolol malate was prepared by dissolving 5mg of Timolol maleate in methanol: water (50:50) to get a solution containing 500µg/ml of Timolol maleate. The working standard solution of Bimatoprost was prepared by diluting the appropriate volume of Bimatoprost stock solution with the diluent to get a solution containing 40µg/ml of Bimatoprost. The working standard solution of Timolol maleate was prepared by diluting the appropriate volume of Timolol maleate stock solution with the diluent to get a solution containing 50µg/ml of Timolol maleate. Binary mixture of Bimatoprost and Timolol maleate was prepared by transferring appropriate volume of standard stock solutions to 100ml volumetric flask and diluting with the diluent to get a solution containing 40µg/ml of Bimatoprost and 50µg/ml of Timolol maleate[3,4].

Preparation of sample solution:

5ml of the ophthalmic solution containing 4mg of Bimatoprost and 5mg of Timolol maleate was transferred into a 100 mL volumetric flask, 60mL of diluent was added and sonicated for 25 min. Further the volume was made up to the mark with diluent. The resulting mixture was filtered through 0.45µ membrane filter. The filtrate thus obtained containing 40µg/mlof Bimatoprost and 50µg/ml of Timolol maleate was used for the analysis5,6.

Analysis of marketed formulation:

Assay of marketed formulation containing 4mg of Bimatoprost and 5mg of Timolol maleate was prepared by preparing the sample solution as described in the preparation of sample. Six injections of the above prepared and standard solutions were injected and the peak areas were determined. The assay of commercial sample was calculated by comparing the areas of standard and sample peaks7,8.

Validation of the method:

Calibration curve (linearity of the HPLC method):

Linearity of Bimatoprost was established by injecting triplicate standard solution preparedby diluting different aliquotes of standard stock solution with the diluent to get the concentration range of 10- 60µg/ml for Bimatoprost. Linearity of Timolol maleate was established by injecting triplicate standard solution prepared by diluting different aliquots of standard stock solution with the diluent to get the concentration range of 12.5-75µg/ml for Timolol maleate[9,10,11,12].

Accuracy: (Recovery)

Accuracy of the method was studied by recovery experiments using standard addition method at three different levels (50%, 100% and 150%). The known amounts of standard solutions containing Bimatoprost (20, 40 and 60µg/ml) and Timolol maleate (25,50and 75µg/ml) were added to prequantified sample solution to reach the 50,100 and 150% levels. These samples were analyzed and from the difference between peak areas of Bimatoprost and Timolol maleate present in the spiked and unspiked samples. The percent recovery of added sample was determined[33, 34,35].

Timolol maleate Accuracy Preparation 50%: (75µg/ml):

From the above Timolol maleate stock solution 1ml and 0.5ml was pipetted out as standard ppm and added ppm respectively into a 10 ml volumetric flask and then make up the volume with diluents. (Added 25µg/ml +STD50µg /ml) [13,14,15].

Timolol maleate Accuracy Preparation 100%: (100µg/ml):

From the above Timolol maleate stock solution 1ml and 1ml was pipetted out as standard ppm and added ppm respectively into a 10 ml volumetric flask and then make up the volume with diluents. (Added 50µg/ml +STD 50µg/ml)[16,17,18].

Timolol maleate Accuracy Preparation 150% (125 µg/ml): From the above Timolol maleate stock solution 1ml and 1.5ml was pipetted out as standard ppm and added ppm respectively into a 10 ml volumetric flask and then make up the volume with diluents. (Added75µg/ml +STD50µg /ml)[31]

Bimatoprost Accuracy Preparation 50% (60 µg/ml):

From the above Bimatoprost stock solution 1ml and 0.5ml was pipetted out as standard ppm and added ppm respectively into a 10 ml volumetric flask and then make up the volume with diluents.(Added20µg/ml +STD40µg/ml)

Bimatoprost Accuracy Preparation 100% (80µg/ml):

From the above Bimatoprost stock solution 1ml and 1ml was pipetted out as standard ppmand added ppm respectively into a 10 ml volumetric flask and then make up the volume with diluents. (Added40µg/ml+STD40µg/ml)

Bimatoprost Accuracy Preparation 150% : (100µg/ml):

From the above Bimatoprost stock solution 1ml and 1.5ml was pipetted out as standard ppm and added ppm respectively into a 10 ml volumetric flask and then make up the volume with diluents. (Added60µg/ml+STD40µg/ml)

Precision: (Repeatability)

Precision of the assay method was demonstrated by determining the response for six repeatedly injected sample solutions and from the peak areas RSD of mean assay value was calculated[28]

Intraday precision:

Intraday precision was demonstrated by injecting six different sample solutions containing Bimatoprost

equivalent to 40µg/ml and Timolol maleate equivalent to 50µg/ml at different time intervals within the same day and %RSD of mean assay value was calculated27.

LOD and LOQ:

LOD and LOQ of Bimatoprost and Timolol maleate were calculated using the following equations as per ICH guidelines.

LOD= 3.3σ/S LOQ= 10 σ/S

Where σ =standard deviation of responseS = slope of regression equation.

Specificity:

Specificity of the method was studied by injecting blank, standard, placebo and sample solutions.

Forced degradation studies:

For forced degradation studies of Bimatoprost and Timolol maleate, standards were forcedto degrade under acid hydrolysis, alkaline hydrolysis, oxidation, photolytic and thermal stress25,26.

For acid degradation: 1ml each of Bimatoprost and Timolol maleate and binary mixture solutions were transferred to 50ml round bottom flasks separately and 1ml of 2N HCl was added to the flasks and about 35ml of methanol was added and refluxed for 30mts at 60ºC.After 30mts, contents of the flasks were cooled and transferred to 50ml volumetric flask and the final volume was made to 50ml with methanol to get a solution containing 40µg/ml of Bimatoprost and 50µg/ml of Timolol maleate23,24.

For alkaline degradation:

1ml each of Bimatoprost and Timolol maleate and binary mixture solutions were transferred to 50ml round bottom flasks separately and 1ml of 2N NaOH was added to the flasks and about 35ml of methanol was added and refluxed for 30mts at 60ºC. After 30mts, contents of the flasks were cooled and transferred to 50ml volumetric flask and the final volume was made to 50ml with methanol to get a solution containing 40µg/ml of Bimatoprost and 50µg/ml of Timolol maleate21,22.

For oxidative degradation:

To 1 ml of stock solution of Timolol and Bimatoprost, 1 ml of 20% hydrogen peroxide (H2O2)was added separately. The solutions were kept for 30 min at 600c. For HPLC study, the resultant solution was diluted to obtain 50µg/ml & 40µg/ml solutionand10µlwereinjectedintothe system and the chromatograms were recorded to assess the stability of sample19,20.

For thermal degradation:

The standard drug solution was placedinovenat1050c for6h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 50µg/ml & 40µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample17,18.

Photo Stability studies:

The photochemical stability of the drug was also studied by exposing the 100 µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 50µg/ml & 40µg/ml solutions and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample15,16.

3. Results and Discussion

Optimization of the method:

Method was optimized by varying chromatographic parameters like column, mobile phasecomposition, mobile phase PH and flow rate to satisfy system suitability testing. Various columns and mobile phase combinations were tried. A satisfactory separation and good peak symmetry was obtained by using Intersil ODS (150×4.6mm, 5µ) column, Acetonitrile: 0.02M Potassium dihydrogen orthophosphate as mobile phase with gradient technique. Quantification was achieved with PDA detection at 213nm based on peak area.

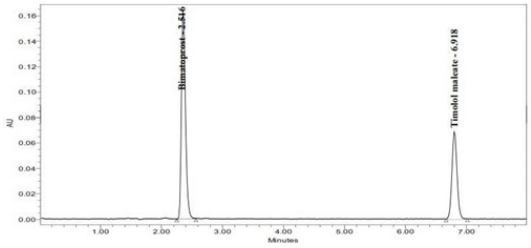

Fig.1Chromatogram of Bimatoprost and Timolol maleate (Bimatoprost 40µg/ml and Timolol maleate 50µg/ml)

Linearity: Regression data is summarized in the table 3.2 which shows a good linear relationship between concentration and peak areas over a concentration range of 10- 60µg/ml for Bimatoprost and 12.5-75µg/ml for Timolol maleate. (Figures 3.2 & 3.3). Thecorrelation coefficient (R^2) was found tobe 0.999 for Bimatoprost and 0.999 for Timolol maleate. The R^2 values for both the drugs were 0.999, which shows that there exist a goodcorrelation between both analyte concentration and response area.

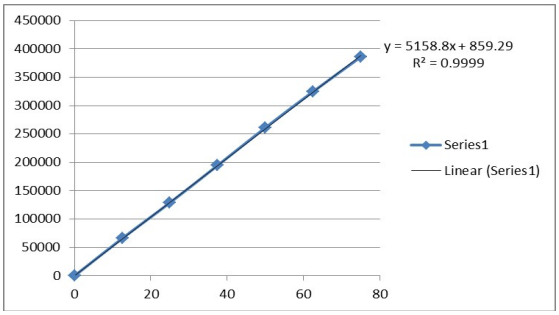

Fig.2 Calibration curve of Timolol maleate

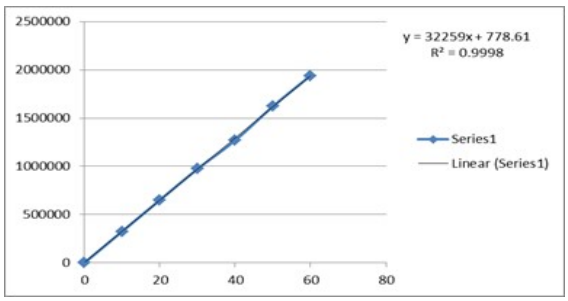

Fig.3 **Calibration curve of Timolol maleate**

Table 1. Regression analysis of the calibration curves for Bimatoprost and Timolol maleate.

Parameter	Bimatoprost	Timolol maleate
Linearity range (µg/ml)	10-60	12.5-75
Regression equation	Y=32259x+778.61	Y=5158.8x+859.29
Correlation coefficient (R^2)	0.9998	0.9999
Y-intercept	778.61	859.29
LOD	0.027069	0.125103
LOQ	0.082028	0.379099

Table 2. Linearity range of Bimatoprost and Timolol maleate

S.NO	Concentration of Bimatoprost(µg/ml)	Response	Concentration of Timolol maleate(µg/ml)	Response	% linearity level
1	0	0	0	0	0
2	10	327495	12.5	65970	25
3	20	643345	25	128832	50
4	30	972118	37.5	194519	75
5	40	1296513	50	260892	100
6	50	1607895	62.5	324486	125
7	60	1948054	75	385492	150

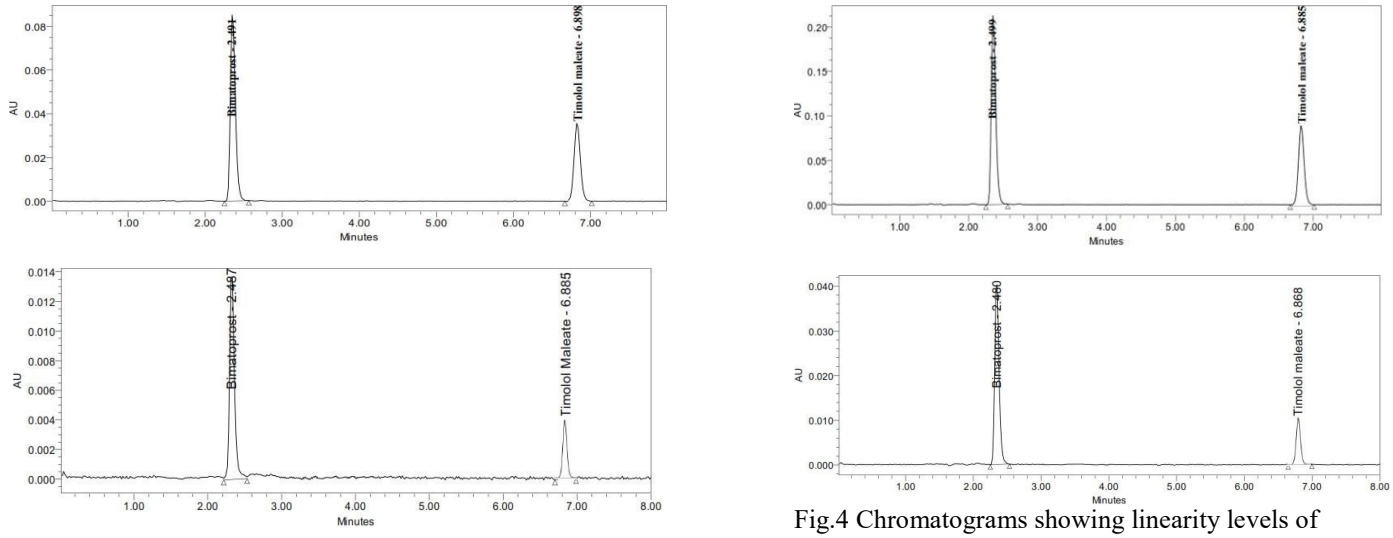

Fig.4 Chromatograms showing linearity levels of a) 25% b) 50% c) 75%d) 100% e) 125% f) 150%

Table 3. Peak purity and peak threshold for various conditions

Stress condition	Bimatoprost		Timolol maleate	
	PA	TH	PA	TH
Standard	0.457	1.926	0.327	0.416
Control sample	0.681	1.490	0.651	1.301
Basic condition	0.472	1.342	0.379	0.711
Oxidation	0.497	1.932	0.383	0.677
Acid	0.477	1.807	0.361	0.487
Photo	0.436	0.787	0.366	0.520
Thermal	0.450	0.837	0.402	0.518

Forced Degradation Studies Results:

The following degradation behavior of the drugs was observed during the HPLC studies.

Acidic conditions: The individual drugs and their combination were heated in 2N HCl for 30mts. No significant degradation was observed for Bimatoprost and Timolol maleate.

Basic condition: The individual drugs and their combination were heated in 2N NaOH for 30mts. No significant degradation was observed for Bimatoprost and Timolol maleate.

Oxidative degradation: No significant degradation was observed in peroxide oxidation.

PDA detection to determine the purity Bimatoprost and Timolol maleate peaks showed purity angle (PA) values and threshold values (TH) as given in the table. The purity angle (PA) value was less than the threshold (TH) values (as evident from the purity plots). The PA value was less than TH values, there by indicating that Bimatoprost and Timolol maleate were free from any co eluting peaks.

4. Conclusion

The developed RP-HPLC method used in routine drug analysis for Bimatoprost & Timolol Maleate in marketed formulation and bulk drug dosage form in pharmaceutical industry

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