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VALIDATED NOVEL STABILITY-INDICATING RP-HPLC METHOD FOR ESTIMATION OF BIMATOPROST AND ITS ISOMER FROM BULK DRUG AND OPHTHALMIC SOLUTION

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ABSTRACT

Bimatoprost (BMT) is novel prostaglandin analogue, chemically related to prostamide F2α useful in eye glaucoma ocular disorder. BMT is optically active due to the presence of chiral centers and exhibit Cis (Z) to Trans (E) isomerism which prompted chiral impurities in bulk drug.These impurities will be carry forward to drug product. In the present study, a simple and economic reversed phase HPLC method was developed for analysis of the BMT and it known chiral impurities in drug substance and drug product. The optimized separation was performed on X-Bridge C18 150mm x 4.6mm, 3.5µ column with isocratic elution by maintaining column temperature at 40 ºC. The mobile phase consisted a simple mixture of water, methanol and acetic acid in the rationof 52:48:01 v/v/v. Detection of analytes was conducted on 210. The stability indicating capability of this method was demonstrated by carrying out forced degradation studies. BMT underwent significant degradation when subjected to acidic and oxidative environment, while BMT is stable in alkali, thermal and photolytic degradation. The degradant did not interfere with BMT and its impurity which is proven by peak purity of each peaks. The performance of this method was validated in accordance to the regulatory guidelines recommended by the International Conference of Homonization (ICH). The stability indicating proposed method in this paper could be applied for process development as well as quality assurance of BMT bulk drug and ophthalmic solution.

KEYWORD: Bimatoprost, Chiral impurities, RP-HPLC, Method development, Validation, Forced degradation.

1. INTRODUCTION

Glaucoma is an ocular disorder with multi-factorial etiology, characterized by slow progressive degenerationof retinal ganglion cells and optic nerve axons. The most important risk factor for glaucoma is rise in intraocular pressure (IOP); leading to progressive visual loss.[1] Bimatoprost (BMT) is chemically 5(Z)-7-{(1R,2R,3R,5S)-3,5-dihydroy-2-[(1E,3S)-3-hydroxy-5-phenyl-1-pentyl] cyclopentyl}-N-ethyl-(5Z)-5-heptenamide. (Fig.1) prostaglandin analogue chemically related to prostamide F2α. BMT believed to decrease (IOP) in humans by increasing the outflow of aqueous humor through both the trabecular meshwork and uveoscleralroutes.BMT is available in market as sterile ophthalmic solution 0.01% and 0.03%.[2,3]

BMT is optically active due to the presence of five chiral center and with the double bond between carbon atom 5 and 6 it exhibit Cis (Z) to Trans (E) isomerism. Based on these two potential chiral impurities associated it. These are 15 (R)-Bimatopost isomer- i.e. 7-{(1R,2R,3R,5S)-3,5-dihydroy-2-[(1E,3R)-3-hydroxy-5-phenyl-1-pentyl] cyclopentyl}-N-ethyl-(5Z)-5-heptenamide(15R-BMT) (Fig.2) and 5,6-Trans Bimatoprost Isomer -7-{(1R,2R,3R,5S)-3,5-dihydroy-2-[(1E,3S)-3-hydroxy-5-phenyl-1-pentyl] -cyclopentyl}-N-ethyl-(5Z)-5-heptenamide (5,6-Trans-BMT)[4]. Since it is associated with BMT which may be increases in storage. According to the current quality requirements the amount of the isomeric impurities need to controlled in bulk drug substances and drug product.[5]

Hence it is necessary to develop the analytical method for estimation of these impurities by a suitable technique. Undoubtedly, high performance liquid chromatography (HPLC) with spectroscopy detection has been considered the most appropriate techniques for identification, and quantification these impurity.[6] After extensive literature survey it was found that no single stability indicating RP-HPLC method was available for determination of these impurities. One of the normal phase HPLC method was reported for determination of these impurities. The reported method has not proven as stability indicating and involved costly non polar organic solvent.[7]

Therefore the main objective of this study was to develop a new, simple, economic, specific and reproducible RP-HPLC stability indicating method for estimation of BMT

and its isomer from bulk drug substances and drug product.

This paper also deals with the validity of the proposed method for the estimation of BMT and its chiral impurities. This proposed method was successfully applied for regular quality control analysis.

2. MATERIAL AND METHODS

Materials and reagents

Sample of Bimatoprost drug product and analytical grade raw materials were purchased from market. The HPLC grade acetonitrile, methanol and acetic acid were purchased from Merck Ltd (India). Double distilled deionized water was used which is generated in lab. All other reagents used in throughout this work were of analytical grade and commercially available.

Instrumentation

HPLC system (Waters Milford, USA) equipped with in built auto-sampler and quaternary gradient pump with an on-line degasser was used. The column compartment having temperature control, photodiode array (PDA) detector (2996) was employed throughout the analysis for detection. Chromatographic data was acquired using Empower software-2 ®.

Chromatographic condition

The analysis was carried out at column temperature 40ºC using X-Bridge C18 150mm x 4.6mm, 3.5µ (Waters) analytical column. The mobile phase composed of mixture of water, methanol and acetic acid in the ratio of 52:48:0.1 v/v/v. The mobile phase was pumped through the column with flow rate of 1mlmin^{-1}. Injection volume 20µL was used in all experiments. The optimum wavelength was selected 210 nm which represents the maximum wavelength for all components. The stressed samples were analyzed using PDA detector covering range from 200-400 nm. The mobile phase was used as diluent for the preparation of working solution which minimizes errors that occur in quantitative separation techniques.

Preparation of standard solutions

Standard solution for assay analysis- A standard solution of BMT was prepared at concentration 180µgmL^{-1} by dissolving about 9 mg BMT working standard in 50 mL volumetric flask with mobile phase by sonication and intermittent shaking.

Standard solution for RS analysis- A standard solution 1.8µgmL^{-1} prepared by diluting 1.0 mL of assay standard stock to 100 mL with mobile phase.

Preparation of test solution for assay and RS

Test solution of bulk sample- prepared a solution at concentration 180µgmL^{-1} by dissolving about 9 mg drug substances into a clean and dried 50 mL volumetric flask by sonication and intermittent shaking.

Test solution of ophthalmic solution- opened five vials pooled the content of each individual vial into a clean and dried glass wide mouth glass vial. Pipette out 3.0 mL of this solution into a 5 mL clean volumetric flask and diluted to the mark with mobile phase and mix well. Filter solution through 0.45 µ nylon filter.

Preparation of system suitability solution

The combine impurity stock solution of 15(R)-BMT and 5,6-Trans BMT was prepared and spiked this solution into respective test solution to achieve the concentration of each impurity1.8µgmL^{-1} (1% with respective to test sample concentration).

Preparation of placebo solution

Opened five vial pooled the content of each individual vial into a clean and dried glass wide mouth glass vial. Pipette out 3.0 mL of this solution to 5 mL clean volumetric flask and diluted to the mark with mobile phase and mix well. Filter solution through 0.45 µ nylon filter.

Preparation of degradation sample solution

Pipette out 3.0 mL of each sample and placebo solutions into two separate 5 mL volumetric flask and exposed to the respective degradation condition. [Table-2] The acid and alkali sample neutralize with each other using same volume and same concentration. These solutions was cooled to room temperature and diluted to volume with mobile phase and mixed well. Filtered solution thorough 0.45 µ filter.

LIST OF FIGURES

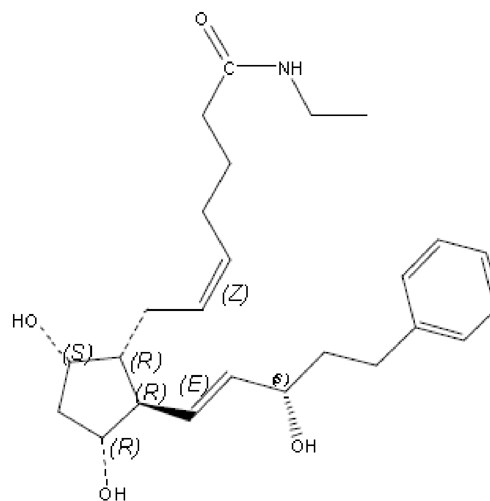

Fig. 1: Structure of Bimaoprost

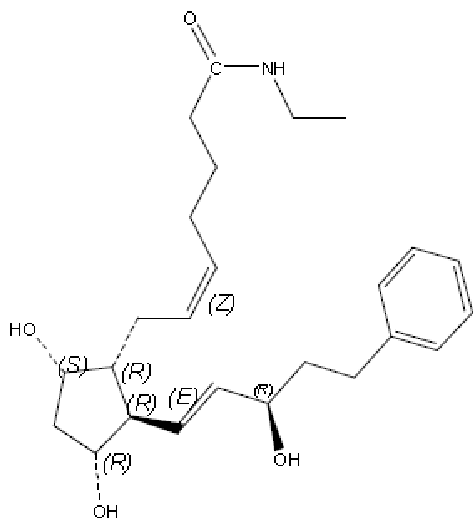

Fig. 2: Structure of 15 (R) - Bimaoprost isomer

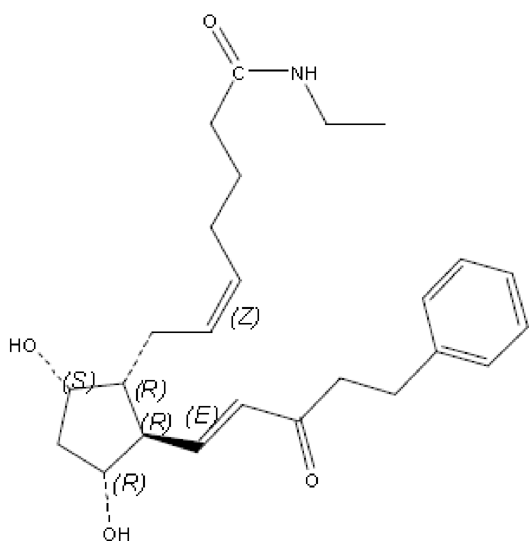

Fig. 3: Structure of 5, 6- Trans Bimaoprost isomer

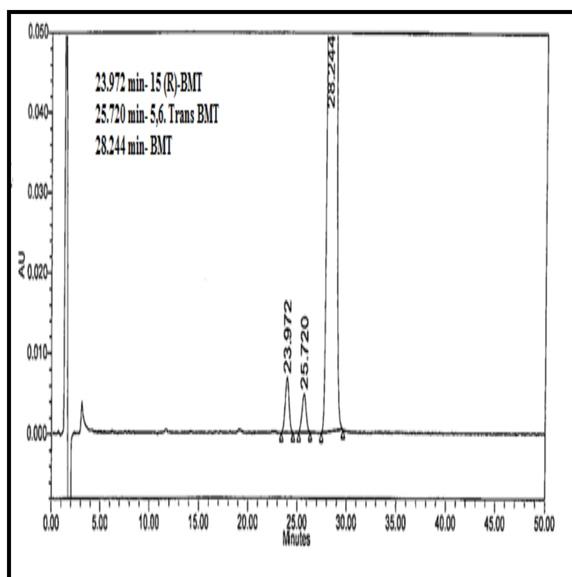

Fig. 4: Chromatogram of test solution spiked with chiral impurities.

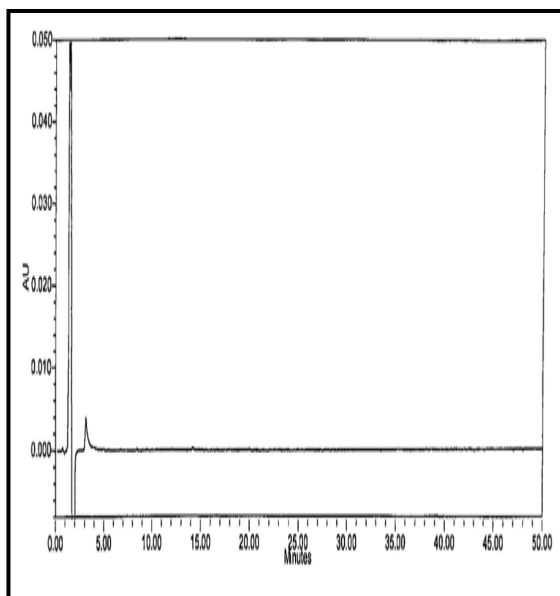

Fig. 5: Chromatogram of placebo solution.

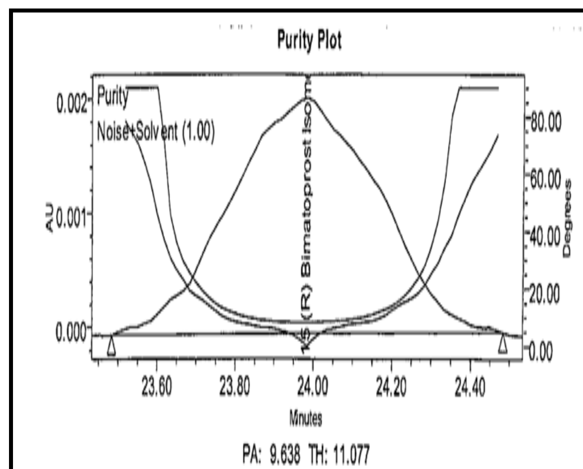

Fig. 6: Peak purity plot of15(R)-Bimatoprost isomer.

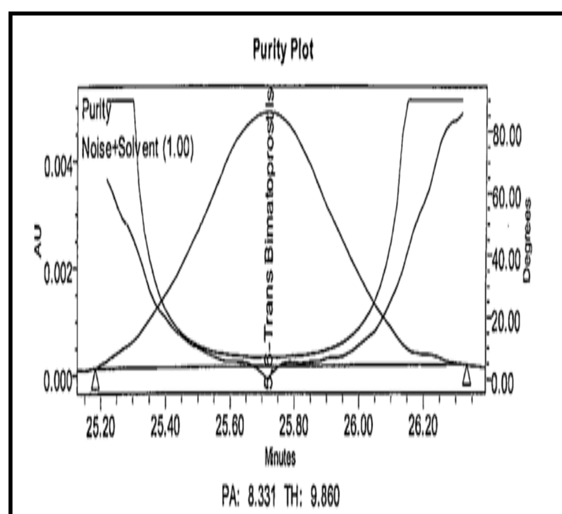

Fig.7: Peak purity plot of 5, 6-Trans Bimatoprost isomer.

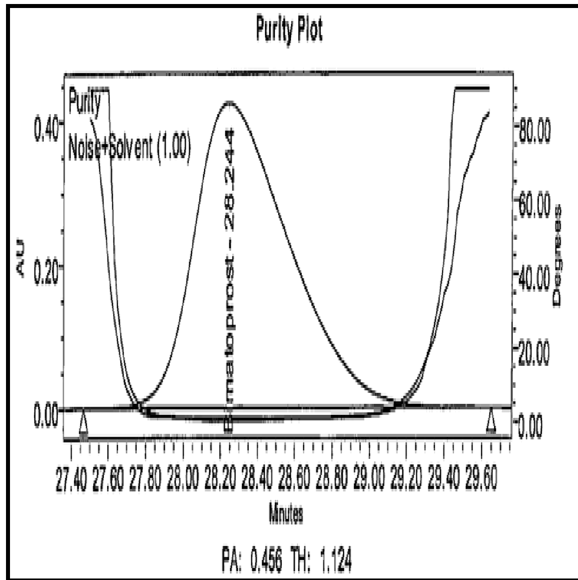

Fig. 8: Peak purity plot of Bimatoprost.

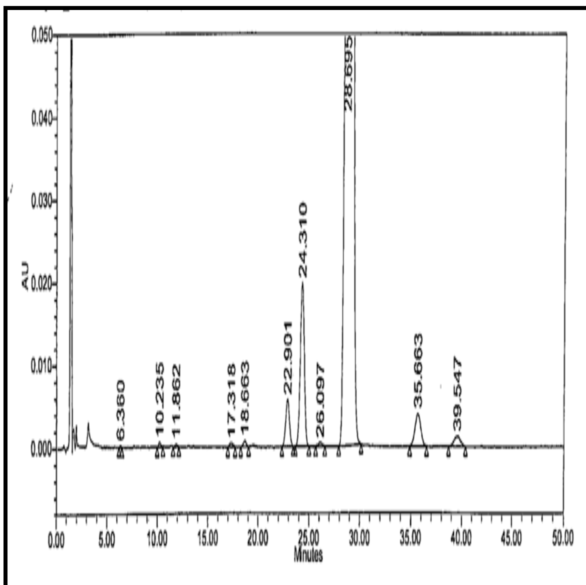

Fig. 9: Chromaogram of acid treated sample.

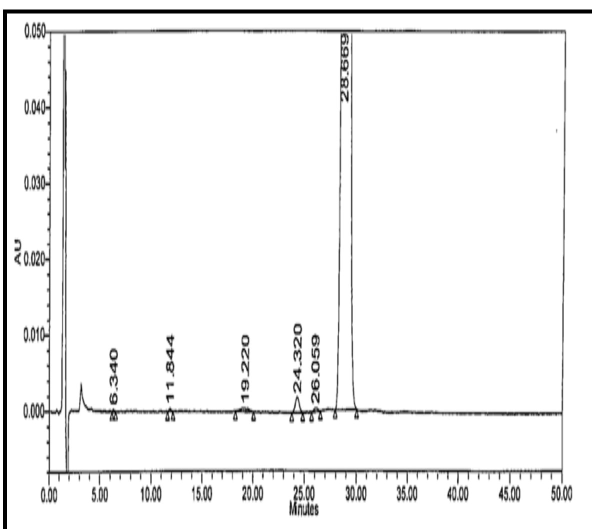

Fig. 10: Chromaogram of alkali treated sample.

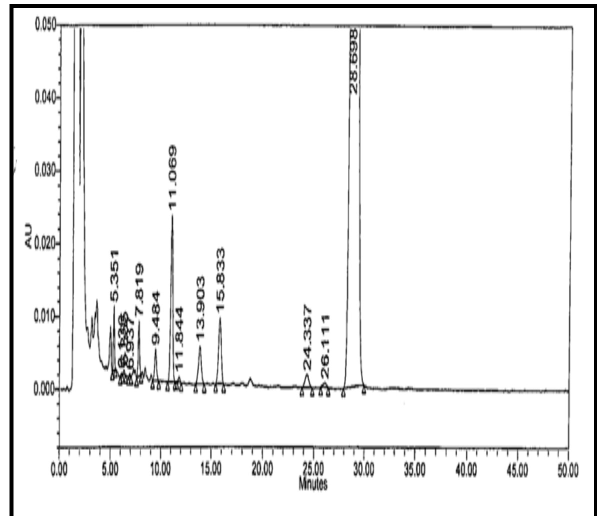

Fig. 11: Chromaogram of peroxide treated sample.

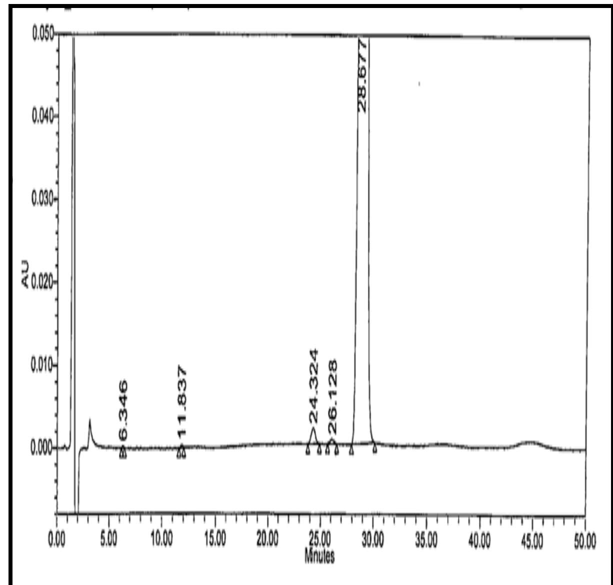

Fig. 12: Chromaogram of thermal treated sample.

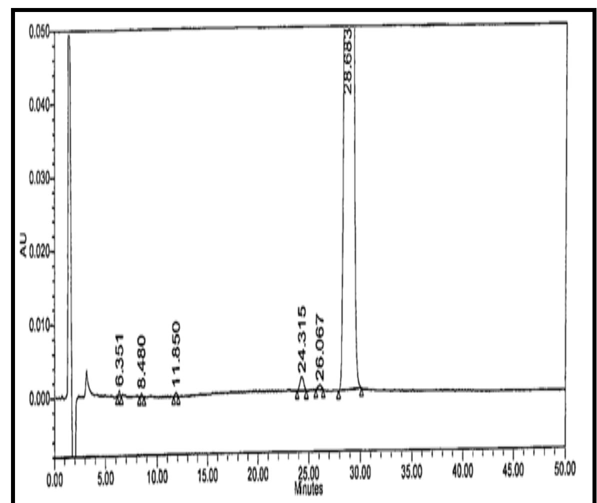

Fig. 13: Chromaogram of photolytic degraded sample.

LIST OF TBALES

Table 1: Results from system suitability.

Parameters	Value (a)		Required Limit
	Assay test	Impurity test	
% f peak area	0.37	0.61	RSD ≤ 2.0 for n≥ 6
Tailing factor (T)	1.20	1.05	T ≤ 2.0
Theoretical plates(N)	10250	18250	Plate N > 5000
Resolution (R)	-	3.1 (b)	R > 2.0

(a)- Mean value of 6 replicates (b) - between 15(R)-BMT and 5,6-Trans BMT.

Table 2: Results of forced degradation studies

Degradation Condition	15(R) BMT (%W/W)	5,6-Trans BMT (%W/W)	Total Unknown(a) (%W/W)	% Mass Balance(b)	Purity Angle value	Purity threshold value
Untreated	0.47	0.13	0.64	_	0.348	1.061
Acid (0.5mL,5NHCl-RT-30min)	4.55	0.13	7.40	96.6	0.299	1.061
Alkali(0.5mL,10NNaOH-60°C-30min)	0.47	0.10	0.78	98.2	0.288	1.059
Peroxide(1.0mL,3% H_2O_2-60°C-30min)	0.39	0.12	5.92	97.1	0.265	1.061
Thermal (60°C-24 hours)	0.49	0.13	0.62	102.1	0.311	1.062
Photo (1.2 million Lux/ hr- 22 hours)	0.41	0.12	0.66	98.2	0.326	1.061

(a)- Sum of known and unknown impurities (b) - Calculated -% assay + % total impurities.

Table 3: Results of precision.

Sample Number	15(R) BMT (%W/W) (a)	5,6-Trans BMT (%W/W)	BMT (%W/W)	
			In Drug substances	In drug product
Set-1	2.33	1.94	98.1	96.5
Set-2	2.23	1.94	96.5	100.2
Set-3	2.24	1.94	97.1	100.3
Set-4	2.24	1.95	98.2	101.1
Set-5	2.23	1.94	96.5	98.5
Set-6	2.25	1.94	97.5	98.6
Mean	**2.24**	**1.94**	**97.3**	**99.2**
SD	1.80	1.80	0.83	1.84
% RSD	**3.18**	**2.93**	**0.85**	**1.85**

Table 4: Results of Linearity, response factor and LOQ, LOD.

Parameters	15(R)- BMT (%W/W)	5,6-Trans BMT (%W/W)	BMT (%W/W)
Slope value	60415	57812	75183
Intercept value	248	-139	-453
Coefficient correlation	0.99995	0.99994	0.99993
RRF value	1.24	1.30	-
LOQ concentration (%w/w)	0.047	0.048	0.067
LOD concentration (% w/w)	0.016	0.016	0.022
S/N for LOQ	47	53	71
S/N for LOD	11	13	21

Table 5: Results of accuracy.

Level	Set	15 (R)- BMT		5,6-Trans BMT		BMT	
		Conc. (µg/mL)	% recovery	Conc. (µg/mL)	% recovery	Conc. (µg/mL)	% recovery
LOQ	1	0.085	102.96	0.086	102.46	0.121	100.10
	2		102.22		100.74		99.98
	3		100.25		103.44		101.05
100%	1	1.764	99.11	1.782	99.02	1.836	99.12
	2		99.51		99.61		98.27
	3		100.10		99.31		97.98
150%	1	2.646	97.70	2.664	99.21	2.682	100.10
	2		98.95		98.62		98.99
	3		99.41		98.82		99.98

Table 6: Results of robustness.

Parameter	Variation	Observed Value	
		% RSD	Resolution (R) [(a)]
Flow rate	0.8 mL/min	0.61	3.10
	1.2 mL/min	0.58	2.81
Column temperature	35°C	0.68	2.72
	45°C	0.58	2.23
Mobile phase composition	46% methanol	0.50	2.92
	50% methanol	0.62	2.21

(a)- Resolution between 15(R)-BMT and 5, 6-trans BMT

3. RESULTS AND DISCUSSIONN

Optimization of chromatographic condition

The first step of method development to selection of suitable column selectivity for the separation of chiral pairs. Due to similar chemical structures and polarities it is critical to separate these impurities with acceptable resolution. Preliminary experiments were performed on different columns included Zorbax C18 150mm x 4.6mm, 3.5µ, X-select C18 150mm x 4.6mm, 3.5µ and X-Bridge C18 150mm x 4.6mm, 3.5µ and Phenmenex Gemini NX C18 150mm x 4.6mm, 3.5µ along with different combination of mobile phases. It was noticed that the studied components have sharp peaks and good separation on X-Bridge C18 150mm x 4.6mm, 3.5µ column. So further optimization was carried out this column.

But the stationary phase is not only the parameter, which can give better chromatography. Mobile phase, pH and organic modifies also plays very important role which leads to the best chromatographic condition.[8] The BMT have high pka (14.35) value and to elute along with chiral impurities within short run time the acidic pH was selected for study. Several mobile phase with different compositions and polarity were examined along with acetic acid for their efficiency in resolution e.g. Water-acetinitrile, water-methanol, potassium phosphate buffer-methanol. The mobile phases with mixture of water, methanol and acetic acid and water, acetonitrile and acetic acid were selected for subsequent investigation as it resulted the best resolution (>5.0) of test components. In water acetonitrile, acetic acid ratio peak shapes of BMT was sharp but resolution between 15(R)-BMT and 5, 6-Trans was not satisfactory. Then trials was undertaken using different ratio of water, methanol and acetic acid to obtained the significant increasing in resolution of degradant from acidic forced degradation studies. Satisfactory separation was achieved using a water, methanol an acetic acid in the ratio 52:48:1v/v/v. By consideration of analysis time; we were tried different organic ratio but impurities resolution was not proper and acceptable. Hence ratio 52:48:01 v/v/v was optimized. The column temperature 40°C and flow rate 1.0 mL min^{-1} were fixed to get minimum column back pressure. The representative chromatograms of this separation of target components were presented in Fig-4. The system suitability parameters were described in Table-1.

Method validation

System suitability

System suitability is the checking of a system to ensure system performance before or during the analysis of unknowns. These tests are integral part of method development and performed to evaluate the behavior of chromatographic system. USP Plate count (N), USP tailing factor (T) and % RSD were evaluated for six replicate injections for BMT peak in standard solutions. The results presented in Table-1.

Specificity

Specificity can be defined as absence of any interference at retention time of peaks of interest and was demonstrated by analysis of test solution spiked with impurities (15R BMT and 5,6-Trans BMT) and by conducting forced degradation studies. The stability indicating supremacy of the method was also proven by these studies.

Interference of Placebo solution and spiked sample solution

A study established the interference of placebo and peak purity of 15 (R) BMT, 5,6- trans BMT and BMT peaks were performed by injecting placebo solution and spiked test solution (spiked known impurities at 1% level with respective with test concentration) into optimized method. Chromatogram of placebo did not show any peak at the retention time of chiral impurities and BMT peak. [Fig-5] This indicate that excipient used in ophthalmic solution do not interference in the estimation of chiral impurities. The peak purity criteria in Empower -2® of all peaks was compiles the acceptance limit viz. purity angle value (PA) less than purity threshold value (TH).[8] This was indicating that no additional peaks were co-eluting with the analyte peaks. [Fig-6-8]

Interference from degradant product

A study was conducted to demonstrate the effective separation of degradant/impurities from BMT peak. The formulation ophthalmic solution was used for study by considering worst case scenario that contains different excipients.The portion of sample and placebo solution were subjected to the ICH recommended forced degradation conditions[9,10] [Table-2]. These stress samples were injected into the HPLC attached with PDA detector by following test condition. The HPLC chromatograms recoded in Fig-9-13. It was found that during the course of stress studied that under alkali,

photo, and thermal degradation no major degradation noted. However the drug was susceptible to degradation in acidic and oxidative medium. The degradation products were satisfactory well separated from known chiral impurities and BMT peak. Moreover, the peak purity of each chiral peak and BMT were checked using peak purity criteria and result indicated that there was no merging of any peak with interested peaks in all stressed samples. These forced degradation samples were also assayed against the control BMT standard. The mass balance was assessed by comparing the decrease in BMT with increasing in all detectable degradation products and found to range of 90-110%. The entire evidence confirmed that this newly developed analytical method was highly specific and selective for intended use. The obtained results tabulated in Table-2.

Precision

The precision of the method was studied taking into account its repeatability and intermediated precision aspects. Precision was determined by injecting six individual preparation of drug substances and drug product ophthalmic solution, each spiked with the both chiral impurities at its specification level i.e. 1% of sample concentration. The % RSD value for content of impurities and BMT were calculated and found to be within < 5.0%. [Table-3] Therefore conclusion could be drawn that this method was sufficiently precise.

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ for chiral impurities and BMT were determined at respective signal to noise ratio (S/N) of 3:1 and 10:1 by serious of diluted solutions with known concentration. The obtained concentration along with their observed S/N ratio was tabulated into Table-4. The results indicated that this method was sufficiently sensitive to carry out the quantification of impurities in bulk drug and ophthalmic solution.

Linearity and Response factor

The linearity of detector response to different concentration was checked for BMT, 15(R)-BMT and 5, 6-Trans BMT using six different concentration level LOQ to 150% of specification level. (1% with respect to BMT test solution concentration 180 μgmL^{-1}). The slope intercept, correlation coefficient value were determined by least square regression analysis. The correlation coefficient obtained was more than 0.999. This was indicating satisfactory linearity of method. The response factor was calculated for 15(R)-BMT and 5, 6-Trans BMT by the formula.

Response factor (RF) = slope value of each BMT / slope value of impurity.

Accuracy

The accuracy of analytical method was determined by measuring recovery through spiking known amounts of impurities in placebo solution. It was carried out in triplicate at three different concentration level ranging from LOQ concentration to 150 % of the specification level. The observed recoveries of all components were within the limit of 80-120%, confirming the accuracy of the determination. The results tabulated in Table-5.

Robustness

The robustness was established by evaluating the influence of minor but significant changes in certain analytical parameters affecting selectivity or affecting quantitative results.

These intended changes includes column temperature ± 5° C, flow rate ± 0.1 mL min^{-1} and change in organic composition 2% absolute. In all deliberated varied chromatographic condition % RSD of peak area of six replicate injections of standard solution was observed less than 5.0%. The resolution of 15(R)-BMT and 5, 6-Trans was greater than 2.0. This indicating that the method is robust enough to maintained reliable results.

4. CONCLUSION

A simple, economic and specific reversed phase HPLC method with UV detection was rationally developed for the separation and quantification of possible impurities in Bimatoprost bulk drug and ophthalmic solution. This method was then fully validated following the regulatory guidelines and found to be specific, linear, precise and robust. Hence the developed HPLC method can be easily applied for the quality control of bulk drug and ophthalmic solution manufacturing of Bimatoprost.

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