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Stability indicating liquid chromatographic method for the determination of Bimatoprost in ophthalmic solutions

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ABSTRACT

A simple and new RP-HPLC method was developed for the estimation of Bimatoprost in ophthalmic solutions using 0.1 % formic acid and acetonitrile (30: 70, v/v) as mobile phase (flow rate 0.6 ml/min and UV detection at 205 nm) using Agomelatine as an internal standard. Shimadzu Model CBM-20A/20 Alite with phenomenex C18 column (250 mm × 4.6 mm i.d., 5 µm particle size) was used for the chromatographic study. Linearity was observed (0.05-15 µg/ml) with regression equation y = 0.1047x + 0.0006 and correlation coefficient 0.9993. Bimatoprost was exposed to forced degradations and the method was validated.

KEY WORDS: Bimatoprost, RP-HPLC, stability-indicating, validation, Agomelatine.

1. INTRODUCTION

Bimatoprost (BMT) is a prostaglandin used for reduction of elevated intraocular pressure in patients. It is also utilised for eyelash treatment (Law, 2010) and eye brow hypotrichosis (Schweiger, 2012) (Beer, 2013). BMT was tested for the growth (Smith, 2012) and management of hypotrichosis of the eyelashes (Fagien, 2015). BMT is chemically, 7-[3,5-dihydroxy-2- (3-hydroxy-5-phenyl-pent-1-enyl)-cyclopentyl]-N-ethyl-hept-5-enamide. Bimatoprost is an ophthalmic topical preparation advised for the control of ocular hypertension, by reducing intraocular pressure. Bimatoprost undergoes metabolism using N-deethylation and glucuronidation pathways. Literature survey reveals that HPLC (Ambhore, 2015) (Suresh Kumar, 2011) and LC/MS (Emilia, 2016) methods were proposed for the assay of cosmetic preparations and pharmaceutical formulations. At present the authors have reported a stability indicating liquid chromatographic method for the determination of Bimatoprost in ophthalmic solutions. Agomelatine is employed as an internal standard and it is an anti-depressant drug.

2. MATERIALS AND METHODS

Chemicals and reagents: Bimatoprost was obtained from Allergan India Ltd. BMT is available in pharmacy as eye drops with brand names CAREPROST, BITOMA, LUMIGAN, LATISSE etc with label claim 0.01% and 0.03%. Acetonitrile, formic acid, sodium hydroxide (AR), hydrochloric acid (AR) and H_2O_2 (AR) were purchased from Merck (India) and all chemicals are of HPLC grade.

The stock solution was prepared by transferring accurately 100 mg of BMT in to a 100 ml volumetric flask in acetonitrile and dilutions were carried out with mobile phase as per the requirement and filtered through 0.45 µm membrane filter prior to injection.

The stock solution of Agomelatine (Internal standard) (AGM) was also prepared by transferring accurately 25 mg of AGM in to a 25 ml volumetric flask in acetonitrile (1000 µg/ml) and 5 µg/ml Agomelatine solution was added as internal standard (IS) after dilution during the entire study.

Instrumentation: Shimadzu Model CBM-20A/20 Alite HPLC system, equipped with SPD M20A prominence photodiode array detector with phenomenex C18 column (250 mm × 4.6 mm i.d., 5 µm particle size) was employed for the entire chromatographic study. Isocratic elution was performed using 0.1 % formic acid and acetonitrile (30: 70, v/v) as mobile phase (flow rate 0.6 ml/min and UV detection at 205 nm) using Agomelatine as internal standard. The chromatographic study was performed at room temperature (25°C±2°C).

Validation:

Linearity: A series of solutions (0.05–15 µg/ml) BMT stock solution with mobile phase along with the internal standard and 20 µL of each of these solutions were injected in to the HPLC system. The peak area ratio of BMT to that of the internal standard (peak area of BMT / peak area of AGM) were calculated from the chromatograms and a calibration curve was drawn by taking the concentration of the BMT solutions on the x-axis and the corresponding peak area ratio values on the y-axis. The limit of quantification and limit of detection measured as described in ICH guidelines Q2 (R1) (ICH guidelines, 2005).

Precision: The intra-day precision of the assay method was evaluated at three concentration levels (50, 100 and 150 µg/ml) and the %RSD was calculated. The inter-day precision study was performed on three different days i.e. day 1, day 2 and day 3 at three different concentration levels (50, 100 and 150 µg/ml) and the % RSD was calculated.

Accuracy: The accuracy of the assay method was evaluated in triplicate at three concentration levels (80, 100 and 120%), and the percentage recoveries were calculated. Standard addition and recovery experiments were conducted

to determine the accuracy of the method for the quantification of BMT in the drug product and the percentage recovery was calculated.

Robustness: The robustness of the assay method was established by introducing small changes in the HPLC conditions which included wavelength (203 and 207 nm), percentage of acetonitrile in the mobile phase (68 and 72%) and flow rate (± 0.05 ml/min). Robustness of the method was studied using 10 µg/ml of BMT.

Forced degradation studies: Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method (ICH guidelines, 2003). All solutions for stress studies were prepared at an initial concentration of 1 mg/ml of BMT and refluxed for 30 min at 80 ºC.

Acidic degradation was performed by treating the BMT solution (1.0 mg/ml) with 0.1 M HCl for 30 min in a thermostat maintained at 80 ºC. The stressed sample was cooled, neutralized with NaOH and then diluted with mobile phase as per the requirement. AGM (Internal standard; 5 µg/ml) was added just before injecting in to the HPLC system. Alkaline degradation was performed by treating the drug solution (1.0 mg/ml) with 0.1 N sodium hydroxide for 30 min in a thermostat maintained at 80 ºC. The stressed sample was cooled, neutralized with HCl and then diluted with mobile phase as per the requirement. AGM (Internal standard; 5 µg/ml) was added just before injecting in to the HPLC system. Oxidation degradation was performed by treating the drug solution (1.0 mg/ml) with 30% H_2O_2 for 30 min in a thermostat maintained at 80 ºC. The drug solution mixture was cooled and then diluted with mobile phase and 20 µl of the solution was injected in to the HPLC system after the addition of internal standard. Thermal degradation was performed by exposing the BMT solution to 80 ºC. The drug solution mixture was cooled, diluted with mobile phase and internal standard was added just before injecting in to the HPLC system.

Assay of ophthalmic solutions: Two different brands procured from the local pharmacy store were transferred carefully in to two different volumetric flasks and extracted BMT with mobile phase. The contents of the volumetric flask were sonicated for 30 min, filtered and diluted with mobile phase as per the requirement. Internal standard was added just before injecting in to the HPLC system and the peak area ratio (BMT/AGM) was calculated from the respective chromatograms.

3. RESULTS AND DISCUSSION

The authors have established a validated stability indicating RP-HPLC method (isocratic mode) for the determination of Bimatoprost using Agomelatine as an internal standard. Mobile phase containing a mixture of 0.1 % formic acid and acetonitrile (30: 70, v/v) with flow rate 0.6 mL/min has shown a sharp peak at 4.732 ± 0.03 min for Bimatoprost and 6.434 ± 0.02 for Agomelatine min during the chromatographic study. The chromatograms of blank and Bimatoprost with internal standard were shown in Figure 2 (a) and (b). Bimatoprost has shown linearity over the concentration range 05–15 µg/mL (Table 1) with linear regression equation $y = 0.2226x + 0.1150$ ($r^2 = 0.9992$) (Figure 3). The LOQ was found to be 0.0461 µg/mL and the LOD was found to be 0.0152 µg/mL.

The intra-day and inter-day precision studies shows the % RSD 0.11-0.28 and 0.13-0.35 respectively. The percentage recovery was found to be 98.83-99.38 % in the accuracy studies with % RSD less than 2.0 (0.32-0.48) (Table 2). The robustness of the method was assessed by exposing the drug solution to different analytical conditions purposely changing from the original optimized conditions. The effects so obtained were summarized to calculate the % RSD and was to be less than 2.0% (0.81-1.01) specifying that the proposed method was robust.

The specificity of the method can be defined from the forced degradation studies. Forced degradation studies were performed in presence of internal standard. The typical chromatograms of the stressed samples were in Figure 4a-4e. Bimatoprost has shown a very slight decomposition i.e. less than 10% during acidic, alkaline, oxidative and thermal degradations. Very small extra peaks were observed during acidic and alkaline degradation studies without interfering with Bimatoprost peak indicating that the method is specific. The percentage drug degradation was less than 10 % in all stressed conditions indicating that Bimatoprost is very much resistant towards all degradations. The present stability-indicating liquid chromatographic method is specific because the drug peak was well separated even in the presence of degradation products and therefore this method can be applied for the determination of BMT in pharmaceutical formulations. The system suitability parameters for the Bimatoprost has shown that the tailing factor was less than 2 (or <1.5-2.0) and the theoretical plates were more than 2000 (Table.3). The proposed method was applied to the marketed formulations and the percentage recovery was found to be 97.80-98.33 (Table.4).

Table.1. Linearity of Bimatoprost

BMT Conc. (µg/mL)	IS Conc. (µg/mL)	peak area BMT	peak area IS	BMT / IS peak area ratio
0.05	5	4402.25	801276	0.005494
0.1	5	8653.575	809654	0.010688
0.3	5	33013	799268	0.041304
0.5	5	46323.5	810341	0.057165
0.6	5	52720	815683	0.064633
1.5	5	142551	822973	0.173215
3	5	277941	811373	0.342556
6	5	499252	823758	0.606066
9	5	796584	815746	0.97651
12	5	1038429	829428	1.251982
15	5	1320675	838759	1.574558

*Mean of three replicates

Table.2. Precision and accuracy studies of Bimatoprost

Conc. (µg/mL)	Intra-day precision			Inter-day precision		
	* measured conc. (µg/mL) ± SD	%RSD	SEM	* measured conc. (µg/mL)± SD	%RSD	SEM
2	2.01 ± 0.01	0.28	0.0032	2.00 ± 0.004	0.19	0.0021
5	4.99 ± 0.01	0.19	0.0055	4.98 ± 0.02	0.35	0.0101
10	9.99 ± 0.01	0.11	0.0064	9.98 ± 0.01	0.13	0.0076

Accuracy

Spiked conc. (µg/mL)	Total theoretical conc. (µg/mL)	*Conc. found (µg/mL)± SD	%RSD	SEM	%Recovery
4 (80 %)	9	8.89 ± 0.00311	0.35	0.1993	98.83
5 (100 %)	10	9.92 ± 0.0477	0.48	0.2752	99.17
6 (120 %)	11	10.93 ± 0.0347	0.32	0.1822	99.38

*Mean of three replicates

Table.3. Forced degradation studies of Bimatoprost

Stress Conditions	*Drug recovered (%)	*Drug decomposed (%)	Theoretical Plates	Tailing factor
Standard drug (Untreated)	100	-	9951.213	1.347
Acidic degradation	92.96	7.04	9545.844	1.354
Alkaline degradation	98.10	1.90	9898.598	1.401
Oxidative degradation	91.62	8.38	10584.704	1.374
Thermal degradation	97.09	2.91	9927.931	1.334

*Mean of three replicates

Table.4. Assay of Bimatoprost in ophthalmic solutions

Formulation	Labelled claim (%)	Amount found* (%)	Recovery* (%)
Brand I	0.01	0.00978	97.80
Brand II	0.03	0.0295	98.33

* Mean of three replicates

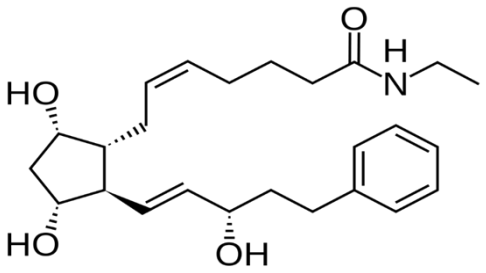

Figure.1. Chemical structure of Bimatoprost

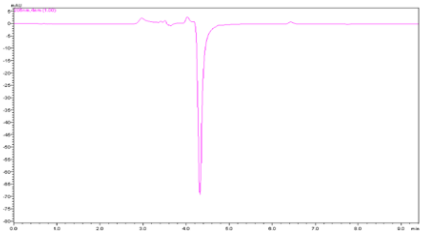

Figure.2a. Typical chromatograms of blank

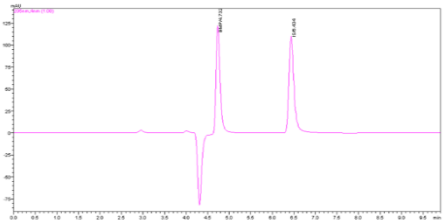

Figure.2b. Typical chromatogram of Bimatoprost (10 µg/ml) with internal standard

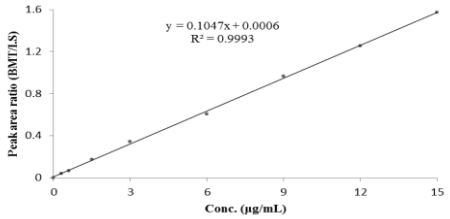

Figure.3. Calibration curve of Bimatoprost

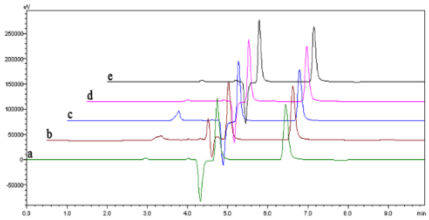

Figure.4. Typical chromatograms of (a) Bimatoprost (10 µg/ml) and acidic (b) alkaline (c) oxidative (d) thermal (e) degradations in presence of internal standard

4. CONCLUSION

The proposed stability-indicating HPLC method was validated as per ICH guidelines and applied for the determination of Bimatoprost in ophthalmic solutions and can be successfully applied to perform long-term and accelerated stability studies. It was observed that Bimatoprost is very much resistant towards forced degradation studies.

5. ACKNOWLEDGEMENT

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