*Research Journal of Pharmaceutical Sciences*_____ __ISSN 2319 – 555X
Vol. **6(1),** 7-10, January **(2017)** *Res. J. Pharmaceutical Sci.*

Reversed phase ultra performance liquid chromatography method for determination of bimatoprost from active pharmaceutical dosage form

Rajan V. Rele* and Sandip P. Patil
Central Research Laboratory, D.G. Ruparel College, Matunga, Mumbai 400016, India
drvinraj@gmail.com

Available online at: www.isca.in, www.isca.me
Received 12th November 2016, revised 28th January 2016, accepted 30th January 2017

Abstract

A reversed phase ultra performance liquid chromatography method was developed and validated for the assay of bimatoprost from active pharmaceutical ingredients. The separation of drug was achieved on Acquity BEH C18 (50mm X 2.1 mm) 1.7µ column. The mobile phase was buffer and acetonitrile in the ratio of 60:40 % v/v. The buffer was used 0.002 M pentane sulphonic acid sodium salt monohydrate .UV detection was performed at 210 nm. The validation of method was done as per ICH guideline using parameters like, specificity, linearity, accuracy, precisions, robustness, solution stability of sample. The developed method was used for analysis of bimatoprost from bulk drug and pharmaceutical dosage form.

Keywords: Bimatoprost, RPUPLC, Pentane sulphonic acid sodium salt monohydrate, Acetonitrile, Methanol.

Introduction

The research works proposes a noble UPLC method for assay of bimatoprost from active pharmaceutical ingredients. Chemically it is 7-[3, 5-dihydroxy-2- (3-hydroxy-5-phenyl-pent-1-enyl) -cyclopentyl]-*N*-ethyl-hept-5-enamide. The prostaglandin analog of bimatoprost was used to control the progression of glaucoma and in the management of hypertension. It is used to control outflow of fluid from the eye. The ophthalmic solution of bimatoprost is used to control of glaucoma, by reducing intraocular pressure. It binds to the prostanoid FP receptor. Literature survey reveals that HPLC[1-2] and LC-MS[3] methods for the determination of bimatoprost. A present research work is developed a new, selective UPLC method for the assay of bimatoprost. This is useful method for the regular analysis of bimatoprost.

Materials and methods

Reagents and chemical: With certificate of analysis standard of bimatoprost was obtained. The analytical grade Pentane sulphonic acid sodium salt monohydrates and the HPLC grade water were used.

Instrumentation: The Water Acquity UPLC system with DAD detector was used. The Empower 3 software was used for quantification of peaks.

An analytical balance was used made of SHIMADZU.

Standard solution: Accurately 45 mg of standard bimatoprost was weighed and to this added about 15 ml of [mixture of water and acetonitrile (50:50 % v/v)]. It was sonicated for 1 minute and diluted up to 50 ml with diluent. Dilute such 5 ml above solution to 50 ml and make up to mark with diluent to obtain concentration 90 µg /ml.

Sample solution: Accurately 45 mg of sample bimatoprost was weighed and to this added about 15 ml of [mixture of water and acetonitrile (50:50 % v/v)]. It was sonicated for 1 minute and diluted up to 50 ml with diluent. Dilute such 5 ml above solution to 50 ml and make up to mark with diluent to obtain concentration 90 µg /ml.

Chromatographic method: The method was developed at 35°C temperature on Acquity BEH C18 (50 X 2.1 mm) 1.7µ column. The mobile phase contains buffer and acetonitrile in the ratio of 60:40 % (v/v) with flow rate adjusted to 0.3 ml /min. The 0.002 M pentane sulphonic acid sodium salt monohydrate solution was as buffer. The 210 nm was a detector wavelength and at 0.8 µl was injection volume.

Method validation: System suitability: Parameter of system suitability parameter was studied by injecting standard solution. Suitability containing number of theoretical plates (N) and tailing factor were shown in Table-1 and it shows that the system is suitable.

Specificity: Specificity of bimatoprost was confirmed by injecting blank, standard. The chromatograms show that the developed method is specific. The standard chromatogram and sample chromatogram are shown in Figure-2 and 3.

Linearity: Linearity of bimatoprost was confirmed by injecting different concentration of known solution. The value of correlation coefficient shows that the method is linear. The results obtained are shown in Table-2.

Table-1: Parameter of system suitability of bimatoprost.

Retention Time in min.	Peak Area of bimatoprost	Percentage Area	Theoretical plates(USP)	Tailing factor(USP)
1.058	359002	100.0	7239	1.2

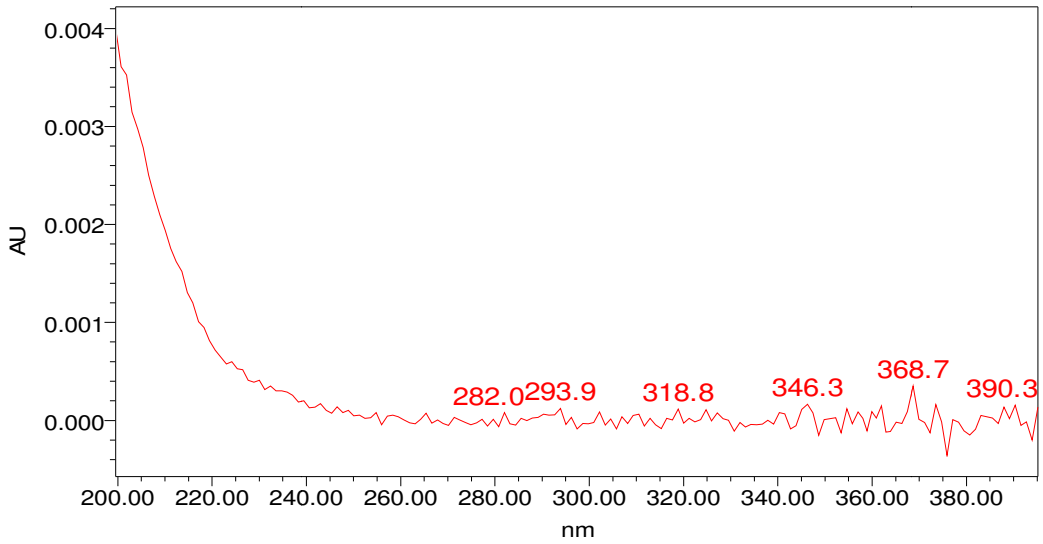

Figure-1: Spectra of bimatoprost.

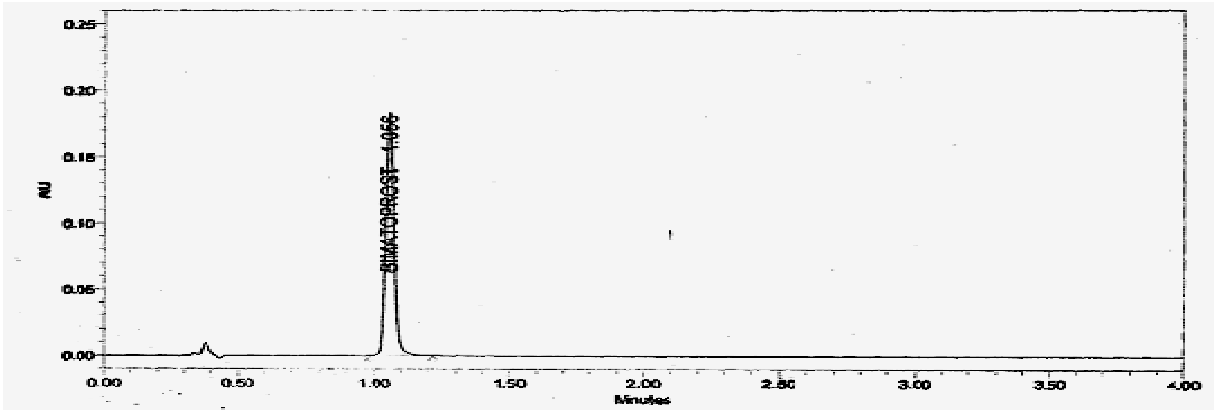

Figure-2: Bimatoprost standard chromatogram.

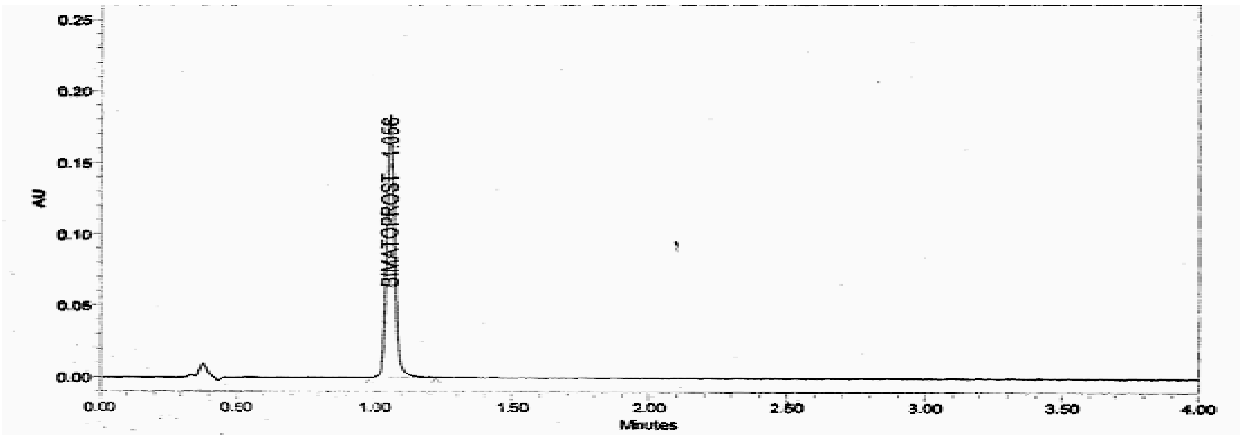

Figure-3: Bimatoprost sample chromatogram.

Accuracy: The recovery experiment is confirmed by injecting three different concentration 80 %, 100 % and 120 %. The recovery was determined by calculating amount of substance found versus amount of substance spiked. The % recovery is tabulated in Table-3.

Precision: In method precision six replicate samples was prepared and calculated relative standard deviation. The results show that the method is precise. Table-4 shows that results of method precision.

Table-2: Results of linearity parameter.

Linearity Parameter	Bimatoprost
Correlation Coefficient (r)	1.0000
% Intercept (y)	-3581.8
Slope (m)	3614.7

Table-3: Accuracy of bimatoprost.

Injection	Stage (%)	Spiked bimatoprost (µg/ml)	Peak Area of bimatoprost	Amount found (µg/ml)	Recovery values in percentage
01	80 %	72.16	290366	72.65	100.68
02			291115	72.84	100.94
03			291110	72.84	100.94
01	100 %	90.20	357290	89.40	99.11
02			357977	89.57	99.30
03			358579	89.72	99.47
01	120 %	108.24	434516	108.72	100.45
02			429524	107.47	99.29
03			429298	107.42	99.24
				Mean	99.94
				Standard Deviation	0.7942
				% RSD	0.79

Table-4: Results of method precision.

Test number	% Assay
Test no. – 1	100.11
Test no. – 2	100.67
Test no. – 3	99.90
Test no. – 4	100.57
Test no. – 5	100.21
Test no. – 6	100.24
Mean	100.33
Standard Deviation	0.3068
% RSD	0.31

Robustness: Changing the small but deliberate variation in method, the robustness parameter was determined. The deliberate variations are: Change in the pump flow rate + 0.02 ml /min, change in concentration of mobile phase + 2 %

Method application: In 10 ml volumetric flask, 1 ml of 0.03% label claim solution of bimatoprost was taken. To this 15 ml [mixture of water and acetonitrile (50:50 % v/v)] was added. It was sonicated for 1 min and make up to volume with diluent to give 300 µg /ml. Dilute 3 ml of above solution in 10 ml volumetric flask and make up with diluent. The identification of analyte peak was confirmed by comparing with standard solution.

Calculated % assay of bimatoprost and results are shown in Table-4. The results were shows that the method meet requirement.

Results and discussion

The revered phase UPLC method developed and validated as per ICH guidelines for the determination of bimatoprost. The value of standard deviation and percent relative standard deviation are within limit. The accuracy study reveals that mean recovery after spiking experiment were found in the range of 99.11 to 100.94.

Conclusion

Hence the developed RP-UPLC method is used for the determination of bimatoprost from bulk drug. The proposed method is selective, repeatable, robust, and cost effective method. Thus this method is recommended for the analysis of bimatoprost from bulk drug and formulation.

References

1. Suresh Kumar S., Natraj K., Asadullah Khan, Kalyan Kumar B. and Venkateshwara Rao J. (2011). Development and validation of RP-HPLC Method for estimation of bimatoprost in Pharmaceutical Dosage Form, *Journal of pharmacy research*., 4(10), 3733-3734.

2. Ambhore N.P., Dandagi P.M., Gadad A.P. and Reddy N.H.S. (2015). Determination of bimatoprost in Bulk and ophthalmic dosage Forms: development and validation of an RP-HPLC method, *Indian drugs,*, 52(12), 49-55.

3. Emilia Marchei, Daniela De Orsi, Carmine Guarino, Maria Concetta Rotolo, Silvia Graziano and Simona Pichini (2016). High performance liquid chromatography Tandem Mass Spectrometry measurement of bimatoprost, latanoprost and travoprost in eyelash enhancing, cosmetic serums. *Cosmetics,* 3(1), 1-4.