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http://www.ijcpa.in

January-March 2016

International Journal of CHEMICAL AND PHARMACEUTICAL ANALYSIS

eISSN: 2348-0726 ; pISSN : 2395-2466

Research Article | Volume-3 | Issue-2 | Article ID: 926

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF BESIFLOXACIN HYDROCHLORIDE IN BULK AND ITS OPTHALAMIC FORMULATION

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Received: 5 January 2016 / Revised: 15 February 2016 / Accepted: 25 February 2016 / Available online : 31 March 2016

ABSTRACT

The present work includes a simple, rapid, accurate and precise isocratic RP-HPLC method for estimation of besifloxacin hydrochloride in bulk and its formulation. The simple, isocratic RP-HPLC method involves separation of besifloxacin hydrochloride on reverse phase C8 oyster column (250 mm x 4.6 mm, 5 µm) with a mobile phase of phosphate buffer (pH 3) :methanol: acetonitrile (50:25:25 % v/v/v). The developed methods were validated successfully according to ICH Q2 (R1) guidelines. The method showed a good linear response with r^2 values of 0.999 . The percentage relative standard deviation for the method was found to be less than two, indicating the method is precise. The mean percentage recovery for RP-HPLC method was between 98.34%-101.56% .Besifloxacin hydrochloride in its opthalamic formulation could be accurately determined with assay value of 98.97%. The developed method is specific, selective and robust. The method could be successfully applied for analysis of opthalamic formulation of besifloxacin hydrochloride.

Keywords *–Besifloxacinhydrochloride ,UV spectrophotometry, RP-HPLC, validation*

1. INTRODUCTION

Besifloxacin hydrochloride[1] is fourth generation flouroquinolone. It is chemically 7-[(3R)-3-Aminohexahydro-1H-azepin-1-yl]-8-chloro-1-cyclopropyl- fluoro- 1,4-dihydro-4-oxo-3-quinolinecarboxylic acid monohydrochloride.

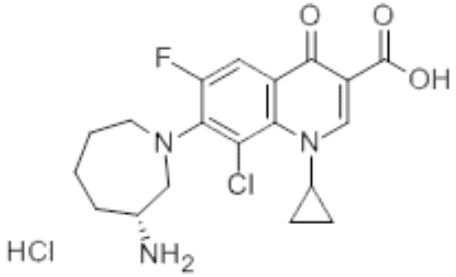

Fig. 1 : Structure of besifloxacin hydrochloride

It is white to pale-yellowish white powder. It is soluble in water, methanol, ethanol and DMSO. pKa value of besifloxacin is 5.65 and 9.67. It has log P value of 4.712. It is marketed as BESIVANCE ophthalmic suspension (0.6% v/v) by Baush and Lomb. It is used in the treatment of bacterial conjunctivitis.

Literature review revealed RP-HPLC method for analysis of solubility, cytotoxic studies [2,3,4] , stability indicating method of besifloxacin hydrochloride[5] . Quantitative estimation of besifloxacin hydrochloride [4] in human tears by liquid chromatography-tandem mass spectrometry has been reported. Enantiomeric impurity in besifloxacin hydrochloride [5] is estimated by chiral HPLC with precolumnderivatisation. Besifloxacin in different simulated body fluids is estimated by UV spectrophotometric method. Our aim is to develop simple, precise , economical and rapid HPLC method development for analysis of Besifloxacin in bulk and formulation.

2. MATERIALS AND METHODS

2.1 Materials

Besifloxacin hydrochloride was purchased from Swapnroop Drugs Private Limited, Aurangabad. BESIVANCE ophthalmic suspension 0.6%w/v (Bausch & Lomb) was purchased from local pharmacy.

Acetonitrile and methanol of HPLC grade were purchased from Rankem, Mumbai.Potassium orthophosphate KH_2PO4 of mol. wt. 136.09 gm/mol was purchased from LobaChemiePvt Ltd, Mumbai). Double distilled water (purified HPLC grade water) was obtained by filtering double distilled water through nylon filter paper 0.2 µm pore size and 47 mm diameter (Pall Life sciences, Mumbai, India).

2.2 Instrumentation

Chromatography was performed on Shimadzu (Shimadzu Corporation, Kyoto, Japan) chromatographic system equipped with Shimadzu LC-20 AT pump and Shimadzu SPD-20AV absorbance detector. Samples were injected through a Rheodyne 7725 injector valve with fixed loop at 20 µl. Data acquisition and integration was performed using Spinchrome software (Spincho biotech, Vadodara).

2.3 Chromagraphic conditions

Chromatographic conditions were obtained using C8 oyster column (250 mm x 4.6 mm, 5 µm) which was maintained at ambient temperature. The analytical wavelength was set at 297 nm and samples of 20µl/ml was injected to HPLC system. The mobile phase was phosphate buffer adhusted to pH 3 with orthophosphoricacid : methanol : acetonitrile in the ratio of 50 :25:25 at flow rate of 1ml/min.

2.4 Preparation of stock solution (1000 µg/ml)

10 mg of besifloxacin hydrochloride was weighed accurately and transferred into a 10 mL volumetric flask containing distilled water. Distilled water was added upto the mark to produce a stock solution containing 1000 µg/mL of besifloxacin hydrochloride.

2.5 Preparation of working standard solution (100 µg/ml)

The solution was prepared by transferring 10 ml of whole stock solution to 100 ml volumetric flask and diluted up to the mark with double distilled water.

2.6 Preparation of mobile phase

A 10 mM phosphate buffer was prepared by dissolving 1.36 g of potassium dihydrogen orthophosphate in sufficient water to produce 1000 ml.The buffer was adjusted to pH 3 with ortho-phosphoric acid. This buffer was filtered through 0.22 µm filter paper and then mixed with acetonitrile and methanol in the ratio of 50 : 25: 25) Then the prepared mixture were sonicated for 5 min and used as mobile phase.

2.7 Preparation of Calibration curve

From the working standard, aliquots ranging from 0.4 ml to 1.4 ml were taken from working standard (100 µg/ml) solution, in 10 ml volumetric flask and diluted to 10 ml with mobile phase to give final concentration of 4, 6, 8, 10, 12, 14 µg/ml of besifloxacin hydrochloride. 20 µl of each concentration were injected in the chromatographic system and was recorded. Calibration graph was constructed by plotting Peak area (mv.S) versus concentration (µg/ml) of each drug and the regression equation was calculated.

2.8 Selection of analytical wavelength

An ideal wavelength is the one that gives good response for the drugs that is to be detected. In the present study drug solutions of 14 µg/ml of besifloxacin hydrochloride was prepared in phosphate buffer. This drug solution was then scanned in the UV region of 200-400 nm and the spectrum was recorded (Fig. 2).The drug shows appreciable absorbance at 297 nm. Prepared calibration standards for both methods were analyzed at 297 nm wavelength.

2.9 Preparation of marketed formulation

1ml of 0.6% aqueous suspension of sample was transferred to 50 ml of volumetric flask and sufficient double distilled water was added and sonicated for 5 min and diluted upto the mark with double distilled water to get the working sample (100µg/ mL). The solution was filtered through whatman filter paper (no. 42). Different volumes of above prepared solution were taken and diluted with mobile phase to get different concentrations of besifloxacin hydrochloride. The above prepared solutions were analyzed by HPLC for the content of besifloxacin hydrochloride using the method described in preparation of calibration curve.

2.10 Method Validation

2.10.1 Linearity and Beer's Range

The linearity of method was evaluated by analyzing prepared concentrations of besifoxacin hydrochloride in range of 4-14 µg/mL.Linear regression equation was obtained over the concentration range ($y = mx+c$). Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated from standard deviation of response and slope of calibration curve.

2.10.2 Precision

Reproducibility of the methods was checked by performing intra-day precision (three times a day) and inter-day precision (repeated triplicates for three consecutive days). Results are expressed in terms of standard deviation and % Relative Standard Deviation (%RSD).

2.10.3 Accuracy

To check the accuracy of the developed methods, recovery studies were carried out from pre-analyzed sample at three different level of standard addition 50%, 100% and 150%. Percentage recovery was the average of three determinations at each standard addition level. Percentage recovery was found to be between 98%-101% which prove that the methods were accurate.

2.10.4 Robustness and Ruggednes

To evaluate robustness of the method few parameters were deliberately varied. The parameters included variation of flow rate, change in pH of buffer, different instruments and acetonitrile of different lots. The average value of % RSD was determination of besifloxacin hydrochloride.

2.10.5 Specificity

The specificity of the method was established through study of resolution factors of the drug peak from the nearest resolving peak and also among all other peaks. A correlation coefficient of

3. RESULTS AND DISCUSSION

RP-HPLC method has been developed for estimation of besifloxacin hydrochloride in bulk and ophthalamicformulation.Chromatographic separation was performed on stainless steel column Oyster C8 (250 X4.6 mm, 5 µm) which was maintained at ambient temperature using mobile phase consisting of phosphate buffer : methanol: acetonitrile (50:25:25) at pH3 at flow rate of 1ml/min. Detection was carried out at 297nm. Besifloxacin hydrochloride obeyed linearity

in the range of 4-14µg/ml.The method was validated and found to be simple,accurate ,sensitive and precise.The percentage recovery of besifloxacin hydrochloride was found to be 99-101%. The method was validated and found to be simple, accurate and precise.

Table 1: Optimized HPLC parameters for Besifloxacin hydrochoride

Method Parameter	Optimized value
Column	Oyster C8 (250mm x 4.6mm, 5µm)
Mobile phase	Phosphate buffer (pH 3):methanol: ACN (50:25:25 % v/v/v)
Retention time	6.027
Detection wavelength	297 nm
Flow rate	1ml/min
Asymmetry	1.067
Temperature	Ambient

Figure 2 shows the overlain chromatogram of besifloxacin hydrochloride by RP-HPLC using the above optimized conditions. Besifloxacin hydrochloride showed a sharp and symmetric peak at 6.07 minutes.

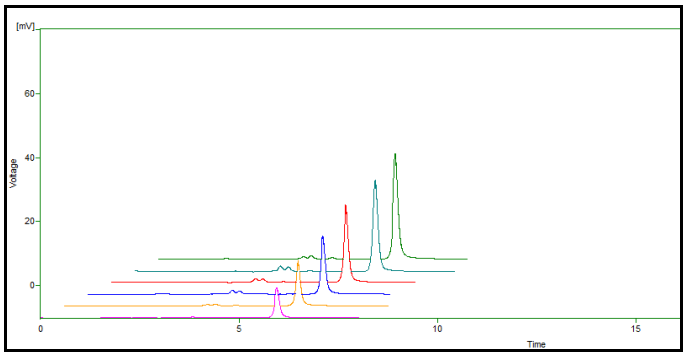

Fig. 2: Overlain chromatogram ofbesifloxacin hydrochloride (4-14 µg/ml)

Table 2: Results of analysis of besifloxacin hydrochloride ophthalmic suspension

Actual conc. of BESI(mg/ml)	Amt* found(mg/ml)	% Assay*
6.63	6.562	98.7%

**Average ±SD (n=3) of experiment*

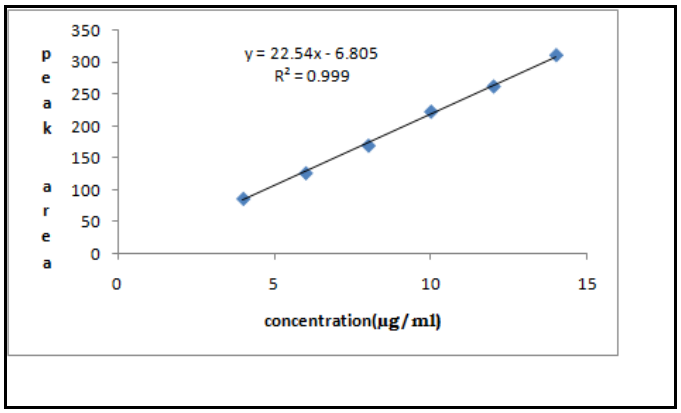

Fig. 3: Calibration curve of besifloxacin hydrochloride (4-14 µg/ml)

Table 2: Summary of validation parameters of besifloxacin hydrochloride

Parameter	RP-HPLC
Analytical wavelength	297 nm
Linearity Range	4-14µg/mL
Slope	22.54
Intercept	6.805
Correlation coefficient (r2)	0.999
Limit of Detection (LOD)	1.005
Limit of Quantification (LOQ)	2.074

Table 3: Results of recovery studies

% spiking	Actual concentration of BESI (µg/ml)	Amount of BESI added	Amount of BESI found	%Recovery*
50%	4	2	1.943	98.34%±1.501
100%	4	4	3.937	99.43%±0.583
150%	4	6	6.093	101.56%±0.433

**Average±SD (n=3) of experiment*

Table 4: Results of intraday and interday precision of besifloxacin hydrochloride

Precision	Standard Deviation	%RSD*
Interday	1.975	0.648
Interday	0.930	0.776

n=6, RSD = Relative Standard Deviation

Table 5: Results of robustness study

Sr. No.	pH(factor)	Retention time	Peak area
1.	2.9	6.095	318.414
2.	3.0	6.015	326.393
3.	3.1	5.987	318.503
	mean ± S.D	6.032±0.056	321.10±0.4581
	wavelength		
1.	296	5.986	320.294
2.	297	6.020	326.401
3.	298	6.027	331.897
	mean ± S.D	6.011 ±0.0219	326.197±0.580
	Solvent		
1.	Acetonitile (Brand 1)	6.129	324.589
2.	Acetonitrile (Brand 2)	5.980	322.478
	mean ± S.D	6.054 ±0.105	323.53±1.049

S.D. : Standard Deviation

Table 6: System suitability parameters of the developed RP-HPLC method

Parameters	Data obtained
Theoretical plates per meter± SD	190821±4016.305
Theoretical plates per column± SD	11382±200.538
Symmetry factor± SD	1.086±0.00273
Retention time(min.) ± SD	6.0100±0.045
Resolution±SD	-

4. CONCLUSION

Simple, specific, accurate and precise RP-HPLC method was developed for besifloxacin hydrochloride. The developed method was validated according to ICH guidelines. The value of %RSD for intra-day and inter-day precision was found less than 2. This value confirms that method is precise. Percentage recovery within 98-102 % for this method shows that the method is accurate and free from the interference of excipients used in formulation.

5. ACKNOWLEDGEMENT

The authors are thankful to Swapnaroop Drugs Pvt Ltd., Aurangabad, for kindlyproviding the sample of API besifloxacin hydrochloride.

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