Research Article   **Open Access**

Design of Experiment Based Validated Stability Indicating Method for Determination of Besifloxacin Hydrochloride in Bulk Drug and Formulation

Sonali S Askarkara, Anvesha V Ganorkara and Krishna R Gupta*

Department of Pharmaceutical Chemistry, Smt Kishoritai Bhoyar College of Pharmacy, India

Received: May 04, 2018; **Published:** May 15, 2018

*Corresponding author: Krishna Gupta, Department of Pharmaceutical Chemistry, Smt. Kishoritai Bhoyar College of Pharmacy, New Kamptee, Nagpur, Maharashtra, India

Abstract

A simple, precise, stability indicating RP-HPLC method was developed and validated for determination of Besifloxacin HCl (Besi) in bulk sample and application of that method for estimation of drug i.A Box–Behnken statistical design with three factors and five levels was employed to optimize the chromatographic conditions. The separation was achieved using solvent system combination using methanol:Acetonitrile: phosphate buffer pH 3(25:25:50). The developed method was validated for linearity, precision, accuracy, limit of detection and limit of quantitation, and robustness in accordance with the ICH guidelines. The method was found linear with regression coefficient of over a wide range of 2.5–15 μg/mL. All the validation parameters were found to be within the acceptance range. The developed method was successfully applied to estimate the amount of Besifloxacin HCl in pharmaceutical formulations.

Abbreviations: BBD: Box-Behnken Design; CCD: Central Composite Design; FFD: Fractional Factorial Design; LOD: Limit of Detection; LOQ: Limit of Quantitation; ANOVA: Application of Analysis of Variance

Introduction

Bacterial conjunctivitis is an inflammation of the conjunctiva, characterised by persistent mucopurlent discharge and redness of the eye [1]. It is generally a self-limiting disease and usually does not cause permanent loss of vision or structural damage to eye. Topical anti-bacterial is used to overcome this disease [2,3]. Bacterial conjunctivitis caused by those bacteria which infect the eye through various sources of contamination. The most common types of bacteria that cause bacterial conjunctivitis include Streptococcus pneumonia, Staphylococcus aureus, Staphylococcus epidermidis, Haemophilus influenza, Moraxella catarrhalis and Corynebacterium spp [4,5]. Aerococcusviridans, CDC coryneform group G, Corynebacterium striatum, Haemophilus influenzae, Moraxella catarrhalis, Moraxella lacunata, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus hominis, Staphylococcus lugdunensis, Staphylococcus warneri, Streptococcus mitis group, Streptococcus oralis, Streptococcus pneumoniae, Streptococcus salivary [6].

Besifloxacin ophthalmic suspension 0.6%w/v was approved by US-FDA in 2009. The formulation uses Duracite® technology to increase the residence time of drug on the ocular [7,8]. Besifloxa cin is a novel fluoroquinolone antibiotic for ocular pathogens that are currently resistant to present fluoroquinolone antibiotics [9]. Besifloxacin (7-[(3R)-3- aminohexahydro -1H-azepin -1-y1] – 8 – choloro -1- cyclopropyl – 6-fluoro -1,4-dihydro -4-oxo-3-quin-olinecarboxylic acid)–(Figure 1) is a novel, chiral synthetic fluo-roquinolone being developed by Bausch & Lomb for the topical treatment of ophthalmic infections. Structurally, besifloxacin has an N-1 cyclo-propyl group, which provides broad- spectrum activ-ity against aerobic bacteria[10]. The main mechanism of action of besifloxacin is the inhibition of DNA gyrase and topoisomerase-IV enzyme that are involved in bacterial transcription replication and separation of chromosomal DNA during cell division [10]. This activity is enhanced by a C-8 chloride substituent in besifloxacin, which also exhibits improved activity against gram-positive bacte-ria relative to older fluoroquinolone while still remaining potent efficacy against anaerobic bacteria [11].

It is white to pale-yellowish white powder. It is sparingly sol-uble in water, methanol, ethanol and soluble in DMSO. pKa value of besifloxacin is 5.65 and 9.67. It has log P value of 4.712. Three different pH of phosphate buffer such as 2.5, 3.0 and 3.5 were stud-

ied and optimized with DOE. This pH was used in the study for best retention of acidic drug in a reversed-phase system. For best retention, the pH of buffer or the mobile phase should be approx. two pH units below its pKa value. In this pH the drug will be totally unionized (more hydrophobic) which helps for better retention in a reversed-phase system. Literature review revealed RP-HPLC method for analysis of solubility, cytotoxic studies [12-14] stability indicating method of besifloxacin hydrochloride [15]. Quantitative estimation of besifloxacin hydrochloride [14] in human tears by liquid chromatographytandem mass spectrometry has been reported. Enantiomeric impurity in besifloxacin hydrochloride [15] is estimated by chiral HPLC with precolumn derivatisation. Besifloxacin in different simulated body fluids is estimated by UV spectrophotometric method.
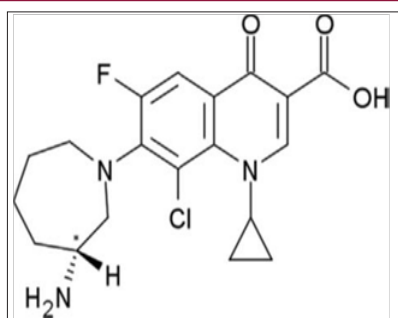

Figure 1: Chemical structure of besifloxacin.

Our aim is to develop simple, precise, economical and rapid HPLC method development for analysis of Besifloxacin in bulk and formulation. The novelty of present method is QbD driven assay method development using solvent system using Acetonitrile:Methanol: Phosphate Buffer pH 3. The QbD based approach provides deeper understanding of the interaction between the factors and their effect on responses. Hence a robust and less variability in method results is obtained. Most of the formulation studies involve only one variant at a time by keeping others as constant. With the help of full factorial design investigators can study the effect of all the factors by varied simultaneously. The factorial design helps to study the effects caused by independent factors and interactions between those self-governing factors. In the present work, three independent factors were used such as Mobile phase (X1), pH of buffer (X2) and flow rate (X3) and five dependent factors were used such as theoretical plate (Y1), tailing factor (Y2), retention time (Y3), Capacity factor (Y4) and Area (Y5).

Materials and Methods

Besifloxacin hydrochloride was a generous gift sample from Glenmark Pharmaceuticals, (Mumbai, India) and used as a working standard. The commercially available formulation of Besifloxacin hydrochloride i.e. Besix ophthalmic suspension 0.6% (Ajanta Pharrma Pvt. Ltd.) were used for Assay. Potassium Di-hydrogen Orthophosphate was of GR Grade, Methanol was of HPLC Grade. All the solutions were prepared with reverse-osmosis, HPLC grade double distilled water. The HPLC System was model Shimadzu and was composed of a binary pump, a mobile phase degasser, a Prominence diode array detector (SPD-M20A) etc. The mobile phase contains Acetonitrile: Methanol and Phosphate Buffer (25:25:50 v/v) with pH 3 adjusted with 1% solution of orthophosphoric acid, flow Rate of 1.0 mL/min and UV Detection was carried out at 297nm.

Chromatographic separation was performed at ambient temperature on an ACE C18 Column (150 × 4.6 mm, 5μ). The Injection volume was 20 μL. Prior to injection of the drug solution, the column was equilibrate for some time with the mobile phase flowing through the system. The data were acquired, stored and analyzed with Design Expert Software Ver 7.0.

Preparation of Standard Stock Solution

Weighed and transferred accurately about 6.63 mg of Besifloxacin hydrochloride standard in a 25mL volumetric flask, 15 mL of methanol was added, sonicated to dissolve and diluted up to the mark with methanol (240μg/mL). 1 mL portion of this solution was further diluted to 10 mL with methanol (24μg/mL). 2 mL portion of this solution was again further diluted to 10 mL with methanol to get final dilution of4.8μg/mL.

Preparation of Sample Solution

An accurately weighed quantity about 1mL of marketed suspension was transferred in to 25mL of volumetric flask. 15 mL of methanol was added and sonicated for 10 min, diluted to volume with diluent. A 1mL of this solution was further diluted to 10mL with methanol (24μg/mL). 2mL portion of this solution was again further diluted to 10 mL with methanol to get final dilution of 4.8μg/mL. Chromatograms of working Standard and sample of Besifloxacin hydrochloride are shown in (Figures 2 & 3) respectively.
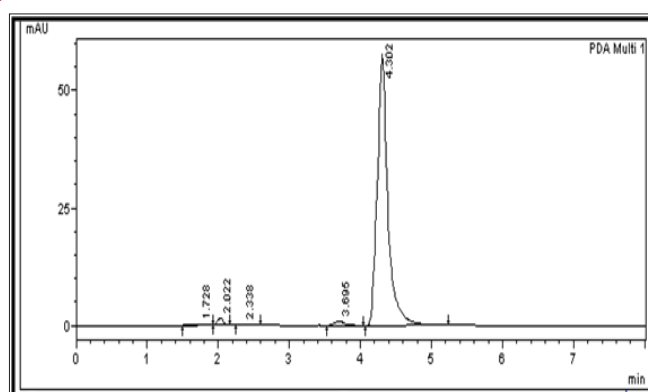

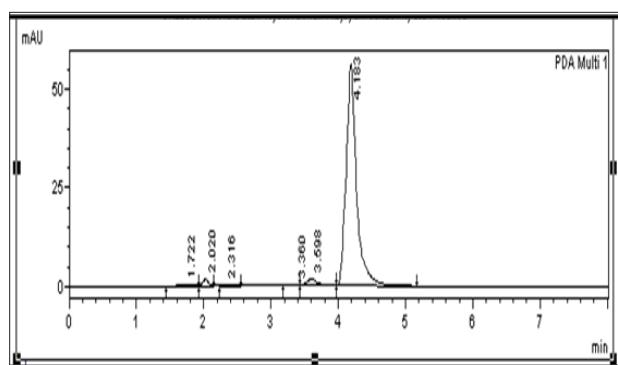**Figure 2:** Chromatogram of working standard of Besifloxacin HCl.

Figure 3: Chromatogram of Besifloxacin HCl Sample.

Method Development and Experimental Design

A novel HPLC Method was developed using the mobile phase composition containing Acetonitrile:Methanol:Phosphate Buffer

(pH 3) for achieving chromatographic separation. The Experimental Design Optimization method along with statistical analysis of data performed by Design-Expert 7.0 software, Full Version, using the Box-Behnken Design (BBD). The BBD has a advantage of optimization for experiments using 3k-factorial design (where, k=1,2,3......) having a least three dependent variables or factors and more than one response as compared to other experimental designs such as Central Composite Design (CCD) and Fractional Factorial Design (FFD). The ratio of mobile phase (Acetonitrile:Methanol: Phosphate Buffer) composition from 25:25:50% v/v, Flow Rate between 0.8-1.2 mL/min and pH between 2.5-3.5 indicating three level was fed in the software and a total of 17 runs were obtained using the design.

Model Design Optimization

The significance of model so obtained was evaluated by two ways i.e. ANNOVA method and Good fit evaluation.

a)   ANOVA: ANOVA is a statistical method based on F-test to estimate the significance of model. It involves subdividing total variation into variation due to Residual error, Main effects and Interactions.

b)   Main Effects (lack of fit): The Lack of Fit is one of the components of partition of the sum of squares in an ANOVA which can tell that proposed model is fit or not.

Estimation of Besifloxacin in Pharmaceutical Dosage Form

The developed HPLC Method was used for determination of Besifloxacin in Pharmaceutical Formulations. Marketed Formulations of Besifloxacin HCl Suspension 0.6%w/v strength was procured from local pharmacy were evaluated for the amount of Besifloxacin present in the formulation. 1mL of Besifloxacin HCl suspension equivalent to Besifloxacin 6 mg was transferred in 25 mL of volumetric flask, 15mL of methanol was added, sonicated for 10 min and volume made up to the mark with diluent. Transferred 1 mL of this solution into 10 mL of volumetric flask and volume made up to the mark with methanol. 2 mL portion of this solution was again further diluted to 10 mL with methanol to get final dilution of 4.8μg/mL. The representative chromatogram of sample was recorded. The Chromatograms were obtained for Besifloxacin in marketed formulation is represented in Figure 3.

Method Validation

The method was validated for

System Suitability

The System Suitability was assessed by six replicate analysis of 4.8μg/mL concentration of Besifloxacin.

Linearity

The Linearity of the method was determined by diluting the standard stock solution to 2.5-15μg/mL.

Accuracy

Accuracy of the method was determined from the recovery of besifloxacin through 4.8μg/mL solution spiked with 80, 100 and 120% extra quantity of Besifloxacin.

Precision

Precision was assessed by the measurement of Intraday precision (repeatability) through the assay of three different concentrations on besifloxacin (2.5-15μg/mL) at different time intervals in the same day and Interday precision by repetition for five days as per ICH Guidelines.

LOD and LOQ

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined from Slope (S) of the Linearity plot and Standard Deviation of the response to the blank sample by formula,

$$\text{Limit of Detection (LOD)} = \frac{3.3\ \sigma}{S}$$

$$\text{Limit of Quantitation (LOQ)} = \frac{10\ \sigma}{S}$$

Solution Stability

The solution stability study was carried out with sample besifloxacin HCl solution. The solution stability study was performed at Room temperature for 24h and in the refrigerator for 24h. After the specified period results were analyzed.

Results and Discussion

The Suitability of Mobile Phase combination, Flow rate and pH was decided on the basis of Linearity, Assay, System Suitability, lesser time required for analysis (Retention Time), Peak Parameters and ease of preparation. Out of several tried combinations as suggested by BBD, the mobile Phase Composition of Acetonitrile:Methanol-Phosphate Buffer (25:25:50% v/v), pH 3 and Flow Rate 1.0mL/min showed efficient chromatographic separation of (4.8μg/mL) and Retention time (Rt) at 4.3min.

Experimental Design

A 32-factorial design using BBD was applied for observing the effect of Three independent factors such as Mobile Phase Composition (% v/v of ACN: Methanol) (X1), Flow Rate (mL/min) (X2) and pH (X3) on three responses such as Theoretical Plates (Y1), Tailing factor (Y2),Retention time (Y3), Capacity factor (Y4), Area (Y5) as parameters for calculation of proposed method. The Chromatographic conditions and Ranges fixed for selected factors are given in Table 1. The sum of total 17 runs were obtained for the fixed variables by selecting a three center repetitions which are generally carried out in order to know the experimental error variance and to test the predictive validity of the model. Each combination of Mobile Phase Composition, Flow Rate, and pH suggested by BBD were finally run on the system; observed for the responses such as theoretical plates, tailing factor, retention time, capacity factor and area are represented in Table 2.

All Experiments were performed in randomized order to minimize the effects of uncontrolled factors that may introduce a bias on the response. Among the various models, the Quadratic model was suggested by the design with the maximum least square

regression for response Y1, response Y2, response Y3, response Y5 and linear model was suggested for response Y4 as compared to other models.The model was examined using Lack of Fit test, which indicated insignificant lack of fit value corresponding with higher p-value as compared to the model F-value. Furthermore, the model was validated by the application of Analysis of Variance (ANOVA) to both the responses and variables to examine the significance of model which showed that both the responses achieved significant differences in their values. From Table 3 the Model F-value of 9.26 implies the model is significant.There is only a 0.39% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob> F" less than 0.0500 indicate model terms are significant.

Table 1: Selection of Independent factors and their Levels.

Factor	Name	Units	Type	Low actual	High actual	Low coded	High coded	Mean	Std. Dev.
A	MP	v/v	Numeric	45	55	-1	1	50	3.43
B	FlowRate	mL/min.	Numeric	0.8	1.2	-1	1	1	0.137
C	pH	pH	Numeric	2.5	3.5	-1	1	3	0.343

Table 2: Box Behenken Design using factors and their responses.

Std. Runs	Sr. No.	Blocks	Volume of org solvent	Ph	Flow Rate (mL/min)	Theoretical Plates	Tailing Factor	R.T.	Capacity factor	Area
6	1	Block 1	55	3	0.8	7396.43	2.147	3.788	0.87	716749
17	2	Block 1	50	3	1	10001.3	1.349	4.925	1.336	718758
15	3	Block 1	50	3	1	10001.3	1.349	4.925	1.336	718758
8	4	Block 1	55	3	1.2	7218.84	2.361	2.799	1.037	467194
12	5	Block 1	50	3.5	1.2	12611.3	1.825	3.238	1.501	456182
11	6	Block 1	50	2.5	1.2	6771.88	2.11	4.368	0.968	681588
5	7	Block 1	45	3	0.8	5494.11	2.378	5.155	1.909	617663
2	8	Block 1	55	2.5	1	5132.88	2.279	3.159	0.982	544377
16	9	Block 1	50	3	1	10001.3	1.349	4.925	1.336	718758
7	10	Block 1	45	3	1.2	7900.03	2.3	4.258	1.926	480484
13	11	Block 1	50	3	1	10001.3	1.349	4.925	1.336	718758
14	12	Block 1	50	3	1	10001.3	1.349	4.925	1.336	718758
9	13	Block 1	50	2.5	0.8	5291.07	2.235	5.073	1.56	660930
4	14	Block 1	55	3.5	1	8571.16	1.825	3.194	0.696	583157
1	15	Block 1	45	2.5	1	5870.49	2.248	6.025	2.553	586592
10	16	Block 1	50	3.5	0.8	14798.7	1.699	4.961	1.479	695440
3	17	Block 1	45	3.5	1	11167.9	1.49	5.311	2.072	591135

Table 3: ANOVA results for response Y1 Theoretical plates.

Sr. No.	Source	Sum of Squares	Df	Mean Square	F values	p-Value Prob>F
1	Model Significant	1.09E+08	9	1.21E+07	9.26	0.0039
2	A-Mp org	5.58E+05	1	5.58E+05	0.43	0.534
3	B-pH	7.25E+07	1	7.25E+07	55.56	0.0001
4	C-flow rate	2.90E+05	1	2.90E+05	0.22	0.652
5	AB	8.64E+05	1	8.64E+05	0.66	0.4426
6	AC	1.67E+06	1	1.67E+06	1.28	0.2954
7	BC	3.36E+06	1	3.36E+06	2.58	0.1524
8	A2	2.83E+07	1	2.83E+07	21.66	0.0023
9	B2	3.19E+05	1	3.19E+05	0.24	0.6363
10	C2	7.01E+05	1	7.01E+05	0.54	0.4873
11	Residual	9.14E+06	7	1.31E+06		
12	Lack of Fit	9.14E+06	3	3.05E+06		
13	Pure Error	0	4	0		
14	Cor Total	1.18E+08	16			

In this case B, A2 are significant model terms.From Table 4 the Model F-value of 37.17 implies the model is significant.There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob> F" less than 0.0500 indicate model terms are significant.In this case B, A2, B2, C2 are significant model terms.Values greater than 0.1000 indicate the model terms are not significant.From Table 5, the Model F-value of 15.08 implies the model is significant. There is only a 0.09% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob> F" less than 0.0500 indicate model terms are significant.In this case A, C, A2, C2 are significant model terms.From Table 6, the Model F-value of 20.45 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob> F" less than 0.0500 indicate model terms aresignificant. In this case A are significant model terms.

Table 4: ANOVA results for response Y2 Tailing factor.

Sr. No.	Source	Sum of Squares	Df	Mean Square	F values	p-Value Prob>F
1	Model significant	2.74	9	0.3	37.17	< 0.0001
2	A-Mp org	4.80E-03	1	4.80E-03	0.59	0.4688
3	B-pH	0.52	1	0.52	63.11	< 0.0001
4	C-flow rate	2.35E-03	1	2.35E-03	0.29	0.609
5	AB	0.023	1	0.023	2.82	0.1368
6	AC	0.021	1	0.021	2.6	0.1506
7	BC	0.016	1	0.016	1.92	0.208
8	A2	0.93	1	0.93	113.81	< 0.0001
9	B2	0.084	1	0.084	10.24	0.0151
10	C2	0.96	1	0.96	117.1	< 0.0001
11	Residual	0.057	7	8.19E-03		
12	Lack of Fit	0.057	3	0.019		
13	Pure Error	0	4	0		
14	Cor Total	2.8	16			

Table 5: ANOVA results for response Y3 Retention time.

Sr. No.	Source	Sum of Squares	Df	Mean Square	F values	p-Value Prob>F
1	Model significant	12.75	9	1.42	15.08	0.0009
2	A-Mp org	7.62	1	7.62	81.1	< 0.0001
3	B-pH	0.46	1	0.46	4.91	0.0623
4	C-flow rate	2.33	1	2.33	24.75	0.0016
5	AB	0.14	1	0.14	1.49	0.2614
6	AC	2.12E-03	1	2.12E-03	0.023	0.885
7	BC	0.26	1	0.26	2.76	0.1408
8	A2	0.88	1	0.88	9.33	0.0185
9	B2	9.06E-03	1	9.06E-03	0.096	0.7653
10	C2	0.92	1	0.92	9.84	0.0165
11	Residual	0.66	7	0.094		
12	Lack of Fit	0.66	3	0.22		
13	Pure Error	0	4	0		
14	Cor Total	13.41	16			

Table 6: VA results for response Y4 Capacity factor.

Sr. No.	Source	Sum of Squares	Df	Mean Square	F values	p-Value Prob>F
1	Model Significant	3	3	1	20.45	< 0.0001
2	A-Mp org	2.97	1	2.97	60.71	< 0.0001
3	B-pH	0.012	1	0.012	0.25	0.6231

4	C-flow rate	0.019	1	0.019	0.38	0.5479
5	Residual	0.64	13	0.049		
6	Lack of Fit	0.64	9	0.071		
7	Pure Error	0	4	0		
8	Cor Total	3.64	16			

Values greater than 0.1000 indicate the model terms are not significant. From Tables 7 & 8, the Model F-value of 8.22 implies the model is significant. There is only a 0.56% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob> F" less than 0.0500 indicate model terms are significant.In this case C, BC, A2, C2 are significant model terms. The model was evaluated for the effect of individual factors on the responses in the form of Counter plots indicating response surfaces of all five responses Y1, Y2, Y3, Y4 and Y5 showed in Figures 4-8 respectively. The counter plot indicates that the effects showed only on four responses i.e Response Y1 (Theoretical plates), Response Y2 (Tailing factor), Response Y3 (Retention time) and Response Y5 (Area) which showed the Quadratic response but response Y4 (Capacity factor) has no effect so it showed the Linear response.
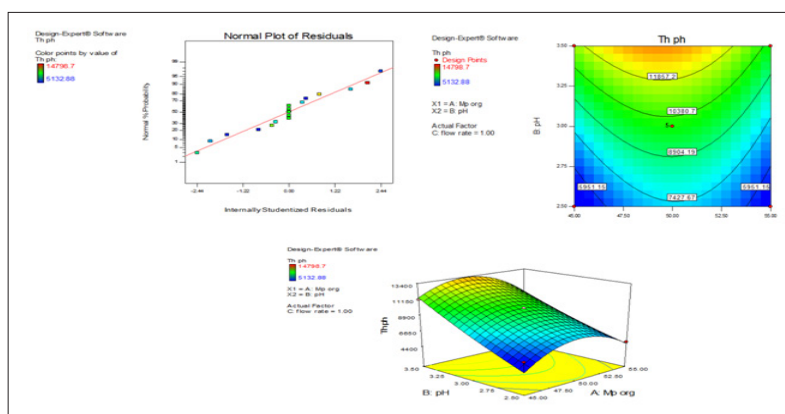

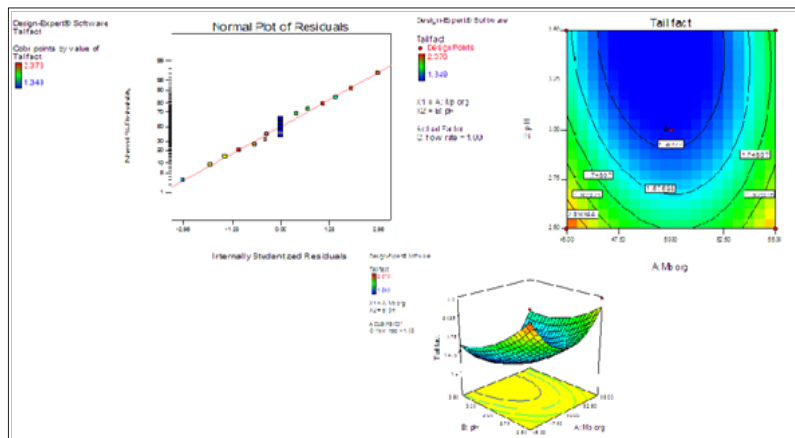**Figure 4:** Normal plot, counter plot and surface response plot for response Y1 Theoretical plates.

Figure 5: Normal plot, counter plot and surface response plot for response Y2 Tailing factor.
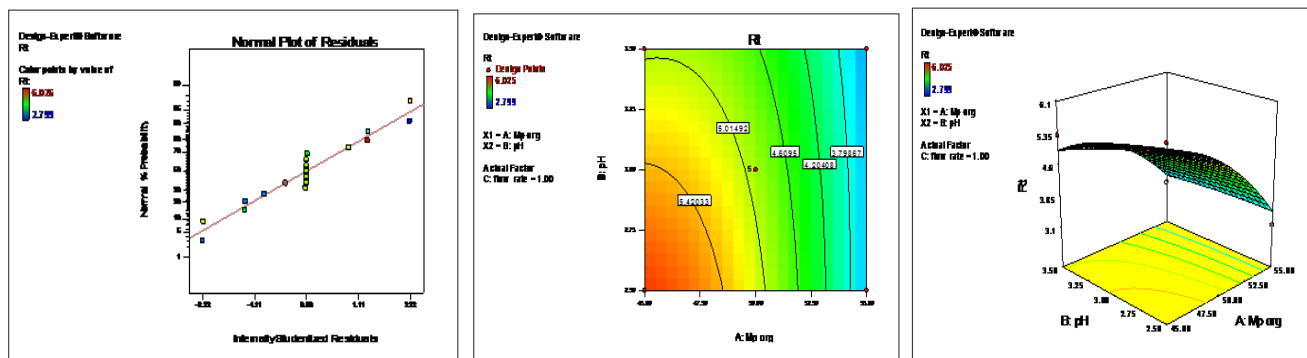

Figure 6: Normal plot, counter plot and surface response plot for response Y3 Retention time.
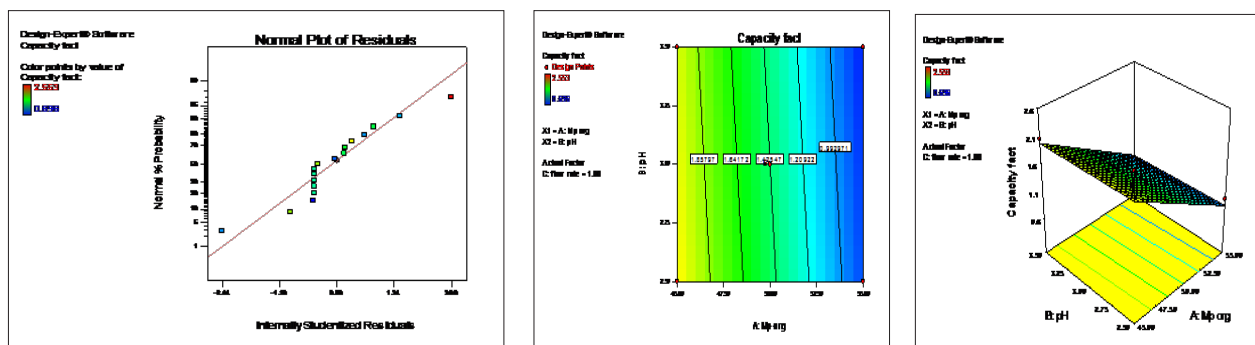
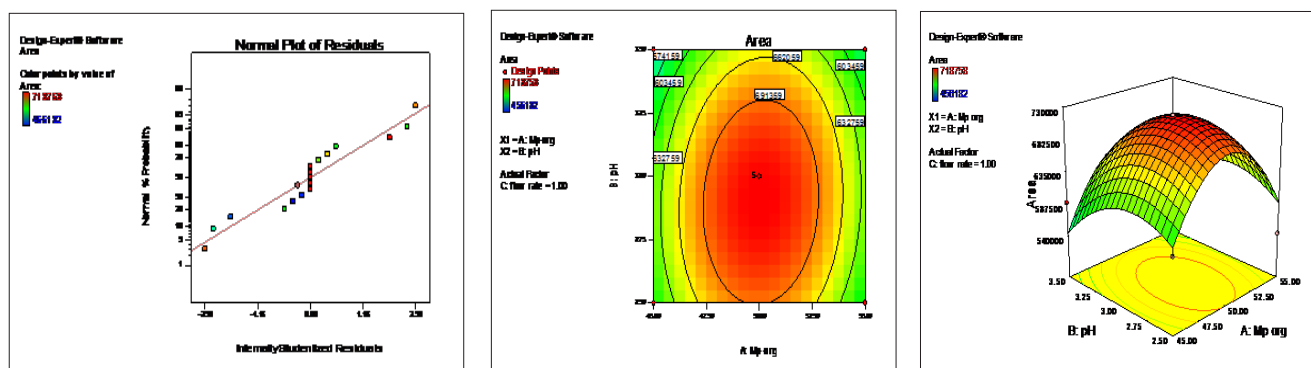**Figure 7:** Normal plot, counter plot and surface response plot for response Y4 Capacity factor.

Figure 8: Normal plot, counter plot and surface response plot for response Y5 Area.

Table 7: ANOVA results for response Y5 Area.

Sr. No.	Source	Sum of Squares	Df	Mean Square	F values	p-Value Prob>F
1	Model significant	1.34E+11	9	1.49E+10	8.22	0.0056
2	A-Mp org	1.58E+08	1	1.58E+08	0.087	0.7762
3	B-pH	2.72E+09	1	2.72E+09	1.5	0.2603
4	C-flow rate	4.58E+10	1	4.58E+10	25.24	0.0015
5	AB	2.93E+08	1	2.93E+08	0.16	0.6998
6	AC	3.16E+09	1	3.16E+09	1.74	0.2287
7	BC	1.69E+10	1	1.69E+10	9.31	0.0186
8	A2	4.02E+10	1	4.02E+10	22.16	0.0022
9	B2	8.42E+09	1	8.42E+09	4.64	0.0682
10	C2	1.07E+10	1	1.07E+10	5.92	0.0452
11	Residual	1.27E+10	7	1.82E+09		
12	Lack of Fit	1.27E+10	3	4.23E+09		
13	Pure Error	0	4	0		
14	Cor Total	1.47E+11	16			

Table 8: Summary of Validation parameters.

Sr. No.	Parameters	Values
1	Linearity range (µg/mL)	2.5-15
2	Regression equation	y=58177x
3	Correlation coefficient	0.999
4	LOD (µg/mL)	0.0116
5	LOQ (µg/mL)	0.2564
6	Accuracy (% recovery)	99.13-100.58% (0.69%)

7	Precision (peak area % RSD)	0.68%
8	Intraday % RSD (n=6)	0.70 %
9	Interday 5 RSD (n=6)	0.90%

Note: LOD = Limit of detection; LOQ = Limit of quantifi cation; RSD = Relative standard deviation

Assay

The absence of additional peaks in the chromatogram indicated no interfearence of the formulation excipient used in the Suspension. The developed method showed good chromatographic separations with a mean percentage recovery of 99.13-100.58%.

Linearity

The data obtained was plotted as peak area against concentration, which showed that the coefficient of correlation (r2) value of 0.999.

System Suitability Study

RSD (%) was determined for peak area was found to be 0.68%.

Accuracy

The method showed the percentage recovery was between 99.13-100.58% and RSD value was 0.69% respectively.

Precision

The method showed good precision and RSD (%) for repeatability and intermediate precision was 0.70 and 0.90% respectively, which were within the NMT 2% limit.

DL and QL

The DL and QL values were found to be 0.0116 and 0.2564 μg/mL indicated the higher sensitivity of the developed method.

Solution Stability

The results of solution stability study after 24h, %RSD was found to be 0.82.

Forced Degradation Studies

Peak purity test results confirmed that the Besifloxacin HCl peak is homogeneous and is pure in all the forced degradation study samples. Thus proposed HPLC method is specific for Besifloxacin HCl.

Conclusion

A robust method for Besifloxacin HCl was developed using a Quality by Design approach. QbD method development software in conjunction with RP-HPLC system automation allows for rapid screening and optimization across a wide range of mobile phases and pH ranges and flow rates while evaluating the effects on factors such as theoretical plates, Tailing factor, retention time, capacity factor and Area on the separation.

Acknowledgment

References

1. Hovding G (2008) Acute bacterial conjunctivitis. Acta Opthalmol 86: 5-17.

2. Tarabishy AB, Jeng BH (2008) Bacterial conjunctivitis: A review for internists. Cleve Clin J Med75: 507-512.

3. Chung CW, Cohen Ej (2000) Eye Disorders: Bacterial conjunctivitis. West J Med 173: 202-205.

4. Sheikh A, Hurwitz B (2006) Antibiotics versus placebo for acute bacterial conjunctivitis. Cochrane Database Syst Rev (2): CD001211.

5. Rose PW, Harden A (2005) Chloramphenicol treatment for acute infective Conjunctivitis in children in primary care: A randomized double blind placebo - Controlled Trial. Lancet 366: 37-43.

6. (2009)Dermatologic and Ophthalmic Drugs advisory committee meeting briefing document. Besifloxacin 0.6% w/v ophthalmic suspension for the treatment of Bacterial Conjunctivitis.

7. (2009) US Deptof Health and Human Services. NDA 22-308. NDA approval.

8. Tepedino ME, Heller WH, Usner DW (2009) Phase III Efficacy and safety study of besifloxacin ophthalmic suspension 0.6% in the treatment of Bacterial Conjunctivitis. Curr Med Res Opin25: 1159-1169.

9. Besivance (package inserts) (2009) Tampa, Fla: Bausch and Lomb Incorporated.

10. Zhang JZ, Ward KW (2008) Besifloxacin a Novel Fluoroquinolone antimicrobial agent, exhibits potent inhibition of pro-inflammatory cytokinines in Human THP-1 Monocytes. JAntimicrob Chemother 61: 111-116.

11. Ward KW(2007)Non-clinical pharmodynamics, pharmacokinetic, safety of BOL- 303224-A, and a novel fluoroquinolone antimicrobial agent for topical ophthalmic use. J Oculpharmacol Ther 23: 243-256.

12. Bausch Lomb (2009) Product monograph-besifloxacin ophthalmic suspension, 0.6% w/v 123400: 1-27.

13. Marcia CNC, Amanda TB, Juliana MMA Talanta Elsevier, Science Direct, 119: pp. 367-374.

14. Jain P, Kar M, Jain DK (2015) International Journal of Pharmaceutical Sciences Letters 5(3): 579-580.

15. Singh CL, Singh A, Santosh BM (2015) World Journal of Pharmaceutical Research 4(15): 2057-2067.

Submission Link: https://biomedres.us/submit-manuscript.php

BIOMEDICAL RESEARCHES

ISSN: 2574-1241
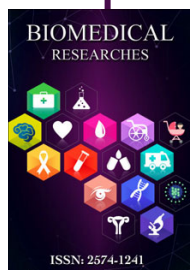
Assets of Publishing with us

- Global archiving of articles
- Immediate, unrestricted online access
- Rigorous Peer Review Process
- Authors Retain Copyrights
- Unique DOI for all articles

https://biomedres.us/