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Development and validation of UV spectrophotometric method for the determination of besifloxacin hydrochloride in bulk drug and finished formulation

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ABSTRACT

Objective: For the quantitation of Besifloxacin Hydrochloride (BSF), a rapid, linear, precise, accurate and robust method using ultraviolet (UV) spectrophotometer in bulk drug and finished formulation (market formulation and developed in situ gel) was developed.

Material and methods: Shimadzu 1700, Kyoto, Japan, double beam UV spectrophotometer, wavelength accuracy ±0.5 nm and a pair of 1.0 cm matched quartz cells were used to measure absorbance of resulting solution. Reference standard and bulk of BSF was purchased from Avanscure Life Sciences Private Limited, Gurgaon, Haryana. Besifloxacin ophthalmic suspension 0.6% w/v (Besix® eye drops), Ajanta Pharma, containing 5 ml of product was procured from a local pharmacy.

Results: The method was found to be linear for Besifloxacin hydrochloride in the range of 4-12 µg/ml with good correlation coefficient (r^2) 0.997), precise with 0.59% RSD, accurate with 98.38-98.74% recovery and robust with 0.54% RSD. The limit of detection and limit of quantitation was found to be 0.589 µg/ml and 1.767 µg/ml respectively.

Conclusion: Proposed method was found to be linear, accurate, precise, robust and suitable for routine quality control analysis for estimation of Besifloxacin hydrochloride in bulk drug and pharmaceutical dosage forms (market formulation and developed in situ gel).

Keywords: UV spectrophotometer, Besifloxacin Hydrochloride, Simulated Tear Fluid (STF).

INTRODUCTION

Besifloxacin (R)-(+)-(3-Amino-2,3,4,5,6,7-hexahydro-1Hazepin-1-yl)-8-chloro-1-cyclopropyl-6-fluoro-1,4,-dihydro-4-oxo-3 quinolinecarboxylic acid hydrochloride (Fig.1). It is an 8-chloro fluoroquinolone with an N-1 cyclopropyl group (Haas et al., 2013). The substituents of the side chain at the 7 position and the chlorine at the 8 position, along with the standard fluoroquinolone core, provide Besifloxacin its unique structure and unique activity profile. Besifloxacin, the latest advanced fluoroquinolone approved for treating bacterial conjunctivitis, is the first fluoroquinolone developed specifically for topical ophthalmic use (Brien et al., 2012). Besifloxacin possesses relatively balanced dual-targeting activity against bacterial topoisomerase IV and DNA gyrase (topoisomerase II). DNA gyrase is an essential enzyme required for replication, transcription and repair of bacterial DNA. Topoisomerase IV is an essential enzyme required for partitioning of the chromosomal DNA during bacterial cell division (Anonymous).

It is fourth generation ophthalmic fluoroquinolone of synthetic origin, approved by the USFDA in May, 2009 for the treatment of bacterial conjunctivitis (Bausch, Lomb, 2009) with marketed name 'Besivance' having 0.6% ophthalmic suspension and sold first in USA under the trade name of Besivance® (Silverstein et al., 2011), (McDonald et al., 2009), (Tepedino et al., 2009), (Zhang et al., 2008), (Ward et al., 2007). The literature reported the estimation of BSF in human tears by tandem mass HPLC (Arnold et al., 2008), bioassay method (Costa et al., 2014), by chiral HPLC (Wang et al., 2012) and UV in different body fluid

(Singh et al., 2015). The method was developed and validated as per International Conference on Harmonization (ICH) guidelines (ICH, Q2A, 1994), (ICH, Q2B, 1996). The present research paper has limit of detection and limit of quantitation 0.589 µg/ml and 1.767 µg/ml respectively however reported UV method shows limit of detection and limit of quantitation 0.72 µg/ml and 2.10 µg/ml respectively (Singh et al., 2015).

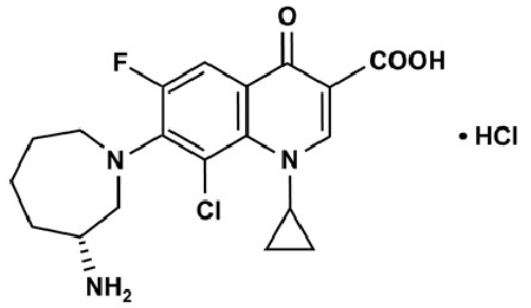

Figure 1. Structure of Besifloxacin hydrochloride

MATERIALS AND METHODS

Double beam UV spectrophotometer, wavelength accuracy ±0.5 nm and a pair of 1.0 cm matched quartz cells. Electronic balance (Mettler Toledo, sensitivity 1 mg). Reference standard and bulk of BSF was purchased from Avanscure Life Sciences Private Limited, Gurgaon, Haryana. Besifloxacin ophthalmic suspension 0.6% w/v (Besix® eye drops), Ajanta Pharma, containing 5 ml of product was procured from a local pharmacy. Double distilled water was used as solvent for experimental purposes. Sodium chloride, Sodium bicarbonate, Methanol Hydrochloric acid and Sodium hydroxide were purchased from S.D. Fine Chem Mumbai, India and Calcium chloride was purchased from CDH, Delhi, India. All chemicals and reagents used for experimental purposes were of analytical grade.

Preparation of simulated tear fluid (STF, pH 7.4)

It was prepared as per the composition given in Table 1 and the pH of the solution was adjusted to 7.4 ±0.05.

Table 1. Compositions of simulated tear fluid (STF, pH 7.4)

Excipients	Quantity (g L^{-1})
Sodium chloride	0.670
Sodium bicarbonate	0.200
Calcium chloride.2H2O	0.008
Purified water (q.s.)	1000 ml

Preparation of standard (Besifloxacin hydrochloride) solution

Accurately weighed 10 mg of *Besifloxacin Hydrochloride* was transferred into 10 ml volumetric flask. It was dissolved with sufficient volume of methanol and diluted up to mark with STF (1000µg/ml). Transferred 0.8 ml of this solution to 100 ml volumetric flask and make the volume with STF.

Preparation of (Besifloxacin hydrochloride) sample solution

Accurately weighed 10 mg of *Besifloxacin Hydrochloride* was transferred into 10 ml volumetric flask. It was dissolved with sufficient volume of methanol and diluted up to mark with STF (1000µg/ml). Transferred 0.8 ml of this solution to 100 ml volumetric flask and make the volume with STF.

Scanning for λmax

The standard solution of *Besifloxacin Hydrochloride* was scanned in the wavelength range of 200- 400 nm using UV spectrophotometer (Fig. 2).

Method validation

The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formulae for the calculation, etc.

The method was validated according to ICH guidelines, Q2 (R1). The method was validated with respect to linearity and range, precision, accuracy, robustness limit of detection (LOD) and limit of quantitation (LOQ).

Linearity and range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

The calibration curve was plotted using the concentration range of 50 to 150% of assay concentration (4- 12 µg/mL).

Preparation of standard stock solution

Accurately weighed 10 mg of *Besifloxacin Hydrochloride* standard was transferred into 10 ml volumetric flask. It was dissolved with sufficient volume of methanol and diluted up to mark with STF to obtain stock solution (1000µg/ml).

Preparation of 50% Linearity Solution

Transferred 0.4 ml of the stock solution in to 100 ml volumetric flask and make up the volume with STF.

Preparation of 75% Linearity Solution

Transferred 0.6 ml of the stock solution in to 100 ml volumetric flask and make up the volume with STF.

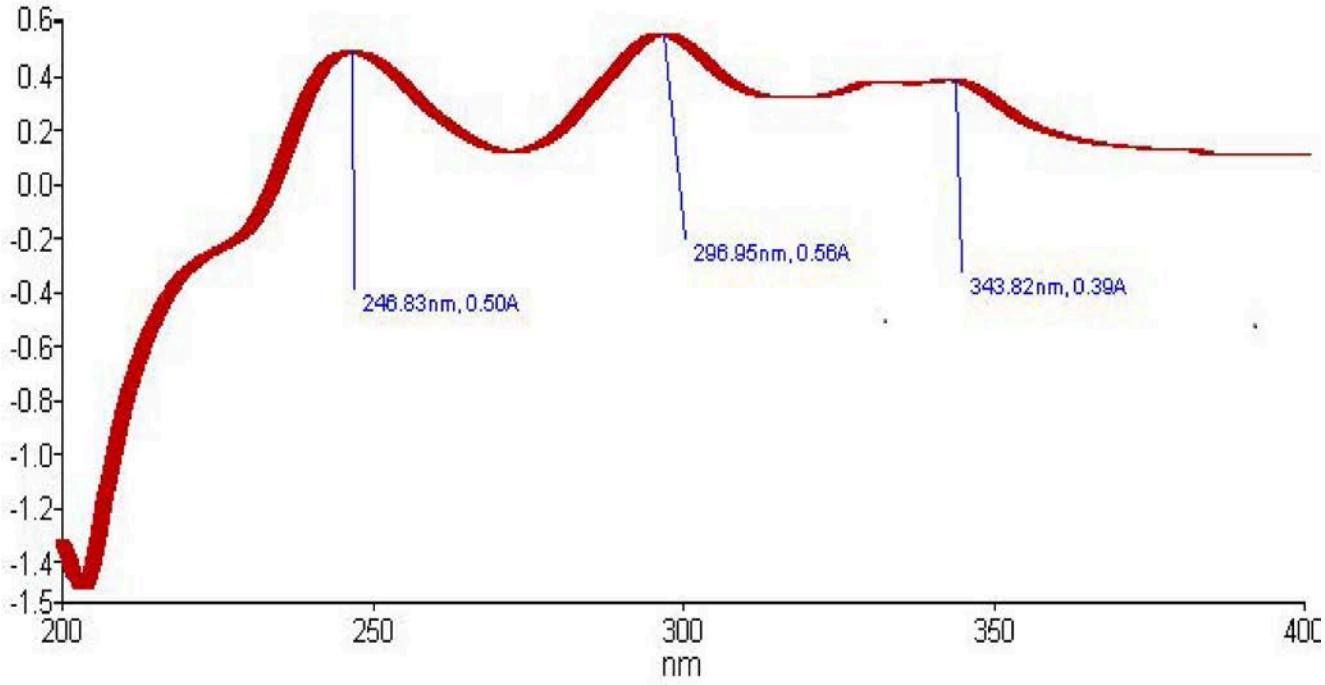

Figure 2. UV Spectra of procured drug.

Preparation of 100% Linearity Solution

Transferred 0.8 ml of the stock solution in to 100 ml volumetric flask and make up the volume with STF.

Preparation of 125% Linearity Solution

Transferred 1.0 ml of the stock solution in to 100 ml volumetric flask and make up the volume with STF.

Preparation of 150% Linearity Solution

Transferred 1.2 ml of the stock solution in to 100 ml volumetric flask and make up the volume with STF.

The absorbance of each solution was determined three times at a wavelength 296 nm. A calibration curve was constructed by plotting absorbance vs. concentration of standard solution and the regression equation was calculated.

System precision

Accurately weighed 10 mg of *Besifloxacin Hydrochloride* standard was transferred into 10 ml volumetric flask. It was dissolved with sufficient volume of methanol and diluted up to mark with STF to obtain stock solution (1000µg/ml). Transferred 0.8 ml of this solution in to 100 ml volumetric flask and make up the volume with STF.

The absorbance of this solution was determined six times at a wavelength 296 nm. The % RSD of absorbance was calculated.

Method Precision

The method precision was assessed by analyzing percentage assay of *Besifloxacin Hydrochloride* in six preparations for intraday precision (within day) and interday precision (different day).

Intraday precision

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Intraday (repeatability) precision was assessed by analyzing percentage assay of *Besifloxacin Hydrochloride* in six preparations for intraday precision (within day). The % RSD of assay was calculated.

Preparation of Method Precision Standard (Besifloxacin Hydrochloride) solution

Accurately weighed 10 mg of *BSF* was transferred into 10 ml volumetric flask. It was dissolved with sufficient volume of methanol and diluted up to mark with STF (1000µg/ml). Transferred 0.8 ml of this solution to 100 ml volumetric flask and make the volume with STF.

Preparation of Method Precision Sample (Besifloxacin Hydrochloride) solution

Accurately weighed 10 mg of *BSF* was transferred into 10 ml volumetric flask. It was dissolved with sufficient volume of methanol and diluted up to mark with STF (1000µg/ml). Transferred 0.8 ml of this solution to 100 ml volumetric flask and make the volume with STF. Similarly prepared five other sample solution.

The absorbance of standard solution was determined three times and absorbances of six sample solutions were determined one time at a wavelength 296 nm. The % RSD of assay was calculated.

Interday (Intermediate) precision

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

Interday (intermediate) precision was assessed by analyzing percentage assay of *Besifloxacin Hydrochloride* in six preparations on second day. The % RSD of assay of 12 preparations (first day and second day) was calculated.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

Accuracy was determined by recovery studies at 80, 100 and 120% of assay sample concentration.

Preparation of Standard Solution

Accurately weighed 8 mg of *Besifloxacin Hydrochloride* standard was transferred into 10 ml volumetric flask. It was dissolved with sufficient volume of methanol and diluted up to mark with STF (800µg/ml).Transferred 1.0 ml of the stock solution in to 100 ml volumetric flask and make up the volume with STF.

Preparation of 80% Recovery Sample Solution

Accurately weighed 6.4 mg of *Besifloxacin Hydrochloride* sample was transferred into 10 ml volumetric flask. It was dissolved with sufficient volume of methanol and diluted up to mark with STF (640µg/ml). Transferred 1.0 ml of this solution in to 100 ml volumetric flask and make up the volume with STF.

Preparation of 100% Recovery Sample Solution

Accurately weighed 8.0 mg of *Besifloxacin Hydrochloride* sample was transferred into 10 ml volumetric flask. It was dissolved with sufficient volume of methanol and diluted up to mark with STF (800µg/ml). Transferred 1.0 ml of this solution in to 100 ml volumetric flask and make up the volume with STF.

Preparation of 120% Recovery Sample Solution

Accurately weighed 9.6 mg of *Besifloxacin Hydrochloride* sample was transferred into 10 ml volumetric flask. It was dissolved with sufficient volume of methanol and diluted up to mark with STF (960µg/ml). Transferred 1.0 ml of this solution in to 100 ml volumetric flask and make up the volume with STF.

The absorbance of each recovery sample and standard solution was determined three times at a wavelength 296 nm. The mean % recoveries of samples were calculated.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

The robustness of method was assessed by analyzing percentage assay of *BSF* in six preparations at different wavelengths (λmax 294, λmax 296 and λmax 298).

Preparation of Standard (Besifloxacin Hydrochloride) Solution

Accurately weighed 10 mg of *Besifloxacin Hydrochloride* was transferred into 10 ml volumetric flask. It was dissolved with sufficient volume of methanol and diluted up to mark with STF (1000µg/ml). Transferred 0.8 ml of this solution to 100 ml volumetric flask and make the volume with STF.

Preparation of Sample (Besifloxacin Hydrochloride) Solution

Accurately weighed 10 mg of *BSF* was transferred into 10 ml volumetric flask. It was dissolved with sufficient volume of methanol and diluted up to mark with STF (1000µg/ml). Transferred 0.8 ml of this solution to 100 ml volumetric flask and make the volume with STF. Similarly prepared five other sample solution.

The absorbance of standard solution was determined three times and absorbances of six sample solutions were determined one time at different wavelength at a wavelength 296 nm. The % RSD of assay was calculated.

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

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines.

LOD = 3.3 × σ/S

LOQ = 10 × σ/S

where

σ = the standard deviation of the response

S = the slope of the calibration curve

Assay of market formulation and developed in situ gel

Preparation of Standard (Besifloxacin Hydrochloride) Solution

Accurately weighed 10 mg of *BSF* was transferred into 10 ml volumetric flask. It was dissolved with sufficient volume of methanol and diluted up to mark with STF (1000µg/ml). Transferred 0.8 ml of this solution to 100 ml volumetric flask and make the volume with STF.

Preparation of Sample Solution of Finished Formulations (Market formulation and developed in situ gel)

Transferred 0.7 ml (4.2 mg) of market formulation into 100 ml volumetric flask. It was dissolved with sufficient volume of methanol with sonicated for 10 minutes and diluted up to mark with STF. Transferred 2 ml of this solution to 10 ml volumetric flask and make the volume with STF. Similarly, developed in situ gel solution sample was prepared.

The absorbance of standard solution and finished formulation were determined at 296 nm in triplicate and percentage assay was calculated by following formula.

$$\% \text{ Assay} = [(AT \times DS)/(AS \times DT)] \times 0.99 \times 100$$

Where:
AT= Absorbance of Test
AS= Absorbance of Standard
DT= Dilution of Test (mg/ml)
DS= Dilution of Standard (mg/ml)

0.99 is the Potency of Besifloxacin Hydrochloride Standard

RESULTS

Linearity and range

The absorbance of the prepared standard solutions (4-12 µg/mL) was determined at **296** nm. Each reading was mean of three determinations. The mean absorbance range (n=3) was found to be 0.2064 to 0.6852 with RSD below 2%. The calibration curve of absorbance versus concentration (µg/mL) obeyed Beer-Lambert's law in the said concentration range with regression coefficient of 0.997 (Fig. 3).

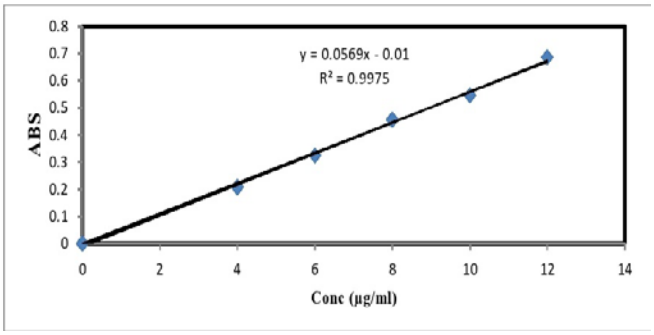

Figure 3. Calibration curve in STF (pH 7.4)

Precision

System, Method and Intermediate Precision

The precision of the system, method and intermediate were determined and results are shown in table 2, 3, 4 and 5. The results were found to be within the limit (<2% RSD).

Accuracy

Accuracy was determined by recovery studies at 80-120% of target concentration. The three samples were prepared at 80, 100 and 120% of the target concentration and these samples were analyzed by the proposed method. The % recoveries of the three concentrations were found to be between 98-102% (98.38-98.74%). The results of recovery are shown in table 6.

Table 2. System precision

Standard Concentration (µg/ml)	Absorbance
8	0.4504
	0.4524
	0.4534
	0.4554
	0.4574
	0.4507
Mean Absorbance	0.4533
Standard deviation	0.0027
%RSD	0.60

Table 3. Intraday precision (Day1)

Standard Solution (µg/ml)	Test Preperations (µg/ml)	Standard Absorbance	Tests Absorbance	% Assay
8	8	0.4504	0.4594	99.71
	8	0.4524	0.4591	99.64
	8	0.4534	0.4535	98.43
	8		0.4555	98.86
	8		0.4559	98.95
	8		0.4599	99.82
Mean		0.4521		99.23
Standard deviation				0.57
% RSD Assay				0.57

Table 4. Interday precision (Day 2)

Standard Solution (µg/ml)	Test Preperati-ons (µg/ml)	Standard Absorbance	Tests Absorbance	% Assay
8	8	0.4513	0.4533	98.02
	8	0.4545	0.4585	99.14
	8	0.4555	0.4525	98.23
	8		0.4568	98.77
	8		0.4599	99.44
	8		0.4567	98.75
Mean Abs		0.4538		98.73
SD				0.54
%RSD Assay				0.54

Robustness

The % RSD of assay at three different wavelengths was calculated and results are shown in table 7, 8 and 9. The result was found to be within the limit i.e. % RSD is less than 2.

Table 5. Precision of Day 1 and Day 2

	1st Day % Assay	2nd Day % Assay
	99.71	98.02
	99.64	99.14
	98.43	98.23
	98.86	98.77
	98.95	99.44
	99.82	98.75
Mean Assay	98.98	
Std	0.59	
% RSD Assay	0.59	

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

LOD and LOQ of the method were determined to be 0.589 and 1.767 µg/ml respectively.

Assay of Market formulation and Developed In Situ Gel

The percentage assay of market formulation and developed in situ gel were calculated and results are shown in Table 1.

Table 6. Accuracy of the method

Standard Solution (µg/ml)	Standard Abs	Level	Amount Added (mg)	Absorbance	Amount Recovered (mg)	% Recovery	Mean % Recovery
8	0.4593	80%	6.4	0.3603	6.3104	98.601	98.74
	0.4598			0.3612	6.3262	98.847	
	0.4512			0.3609	6.3210	98.765	
	0.4593	100%	8.0	0.4533	7.9393	99.241	99.56
	0.4598			0.4585	8.0304	100.379	
	0.4512			0.4525	7.9253	99.066	
	0.4593	120%	9.6	0.5395	9.4490	98.427	98.38
	0.4598			0.5395	9.4490	98.427	
	0.4512			0.5388	9.4368	98.300	

Table 7. Robustness of the method (*n* = 6) at 294 nm.

Standard Solution (µg/ml)	Test Preperations (µg/ml)	Standard Absorbance	Tests Absorbance	% Assay
8	8	0.4512	0.4596	99.24
	8	0.4532	0.4585	99.00
	8	0.4588	0.4594	99.59
	8		0.4560	98.46
	8		0.4550	98.25
	8		0.4599	99.30
Mean Abs		0.4544		98.97
Std				0.52
% RSD Assay				0.53

Table 8. Robustness of the method (*n* = 6) at 296 nm.

Standard Solution (µg/ml)	Test Preperations (µg/ml)	Standard Absorbance	Tests Absorbance	% Assay
8	8	0.4504	0.4594	99.71
	8	0.4524	0.4591	99.64
	8	0.4534	0.4535	98.82
	8		0.4555	98.86
	8		0.4559	98.95
	8		0.4599	99.82
Mean		0.4521		99.30
Standard deviation				0.47
%RSD Assay				0.47

Table 9. Robustness of the method (*n* = 6) at 298 nm.

Standard Solution (µg/ml)	Test Preperations (µg/ml)	Standard Absorbance	Tests Absorbance	% Assay
8	8	0.4512	0.4598	99.44
	8	0.4598	0.4556	98.53
	8	0.4501	0.4522	98.18
	8		0.4595	99.37
	8		0.4591	99.28
	8		0.4593	99.33
Mean		0.4537		99.02
Std				0.53
%RSD Assay				0.54

Table 10. Assay of market formulation and developed in situ gel.

	Standard solution (8 µg/mL)	Market Preparation Absorbance (8.4 µg/mL)	Developed in Situ Gel Absorbance (8.4 µg/mL)	% Assay of Market Formulation	% Assay of Developed in Situ Gel
	0.4531	0.4733	0.4771	98.67	99.46
	0.4523	0.4765	0.4734	99.34	98.69
	0.4514	0.4712	0.4755	98.23	99.13
Mean	0.4523			98.75	99.09

DISCUSSION

For the successful estimation of BSF from marketed and developed in situ gel system, the developed the simple, précised, and accurate ultraviolet spectroscopy analytical method. At lower concentration of linearity range (4-12 µg/ml) the absorbance reaches close to 1 with high regression coefficient of 0.997. It helps in estimation of drug form developed formulation at low concentration. The high regression coefficient showed an excellent correlation between absorbance and concentration (Paudel et al., 2014). The low value of RSD in precision showed there is no interference of the operating conditions, proves high precision of the method (ICH, Q2A 1996). The robustness of the method were found to be within limit i.e., <2% RSD indicates method to remain unaffected by small variations in method parameters. The recovery of BSF was found to be 98.38-99.56%. The LOD and LOQ were found to 0.589 and 1.767 µg/ml respectively.

CONCLUSION

The developed method was found to be very simple, rapid, linear, accurate, precise, robust and economical. This developed UV-method can be used

for the analysis of BSF in simulated tear fluids in bulk, marketed formulations and developed in situ gel system. Thus proposed method will be suitable for the analysis of Besifloxacin hydrochloride in bulk drug and pharmaceutical dosage forms (market formulation and developed in situ gel).

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CONFLICT OF INTEREST

None declared.

REFERENCES

Anonymous. [http://www.rxlist.com/besivance-drug/clinical-pharmacology.htm]

Arnold DR, Granvil CP, Ward KW, Proksch JW. Quantitative determination of besifloxacin, a novel fluoroquinolone antimicrobial agent, in human tears by liquid chromatography-tandem mass spectrometry. Journal of Chromatography B, Analytical Technologies in the Biomedical and Life Sciences. 2008;867:105-10.

Bausch, Lomb. New topical ophthalmic antibacterial for the treatment of bacterial conjunctivitis. Receives FDA Approval of Besivance; 29 May, 2009.

Brien, TP. 2012. Besifloxacin ophthalmic suspension, 0.6%: a novel topical fluoroquinolone for bacterial conjunctivitis. Advances in Therapy. 2012; 29(6): 473-90.

Costa MC, Barden AT, Andrade JM, Oppe TP, Schapoval EE. Quantitative evaluation of Besifloxacin ophthalmic suspension by HPLC, application to bioassay method and cytotoxicity studies. Talanta. 2014;119:367-74.

Haas W, Sanfilippo CM, Hesje CK, Morris TW. Contribution of the R8 substituent to the *in vitro* antibacterial potency of besifloxacin and comparator ophthalmic fluoroquinolones. Clinical Ophthalmology. 2013;7:821-30.

ICH, Q2A, Hamonised Tripartite Guideline. Test on Validation of Analytical Procedures, IFPMA. In: Proceedings of the International Conference on Harmonization. Geneva; 1994.

ICH, Q2B, Hamonised Tripartite Guideline. Validation of Analytical Procedure: Methodology, IFPMA. In: Proceedings of the International Conference on Harmonization. Geneva; 1996.

McDonald MB, Protzko EE, Brunner LS, Morris TW, Haas W, Paterno MR, *et al.* Efficacy and safety of besifloxacin ophthalmic suspension 0.6% compared with moxifloxacin ophthalmic solution 0.5% for treating bacterial conjunctivitis. Ophthalmology 2009;116:1615-1623. e1.

Silverstein BE, Allaire C, Bateman KM, Gearinger LS, Morris TW, Comstock TL. Efficacy and tolerability of besifloxacin ophthalmic suspension 0.6% administered twice daily for 3 days in the treatment of bacterial conjunctivitis: A multicenter, randomized, double masked, Vehicle controlled, parallel group study in adults and children. Clinical in Therapy. 2011;33:13-26.

Singh CL, Singh A, Kumar S, Kumar M, Sharma PK, Majumdar DK. Development and validation of different ultraviolet spectrophotometric methods for the estimation of besifloxacin in different simulated body fluids. Indian Journal of Pharmaceutical Sciences. 2015; 77: 399-404.

Tepedino ME, Heller WH, Usner DW, Brunner LS, Morris TW, Haas W, *et al.* Phase III efficacy and safety study of besifloxacin ophthalmic suspension 0.6% in the treatment of bacterial conjunctivitis. Current Medical Research and Opinion. 2009;25:1159-69.

Wang Z, Wang S, Zhu F, Chen Z, Yu L, Zeng S. Determination of enantiomeric impurity in Besifloxacin hydrochloride by chiral high performance liquid chromatography with precolumn derivatization. Chirality. 2012;24:526-31.

Ward KW, Lepage JF, Driot JY. Nonclinical pharmacodynamics, pharmacokinetics, and safety of BOL303224A, a novel fluoroquinolone antimicrobial agent for topical ophthalmic use. Journal of Ocular Pharmacology and Therapeutics. 2007;23:243-56.

Zhang JZ, Ward KW. Besifloxacin, a novel fluoroquinolone antimicrobial agent, exhibits potent inhibition of proinflammatory cytokines in human THP1 monocytes. The Journal of Antimicrobial Chemotherapy. 2008;61:111-16.