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RP–HPLC Method for the determination of Balofloxacin in Bulk and Pharmaceutical Dosage form

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Abstract: A reverse phase high performance liquid chromatographic method was developed for the determination of Balofloxacin in bulk and Pharmaceutical dosage form. The separation was effected on a Zodiac C_{18} column (150 mm x 4.6 mm; 5µ) using a mobile phase mixture buffer of 0.01M potassium dihydrogen phosphate and acetonitrile in a ratio of 40:60 v/v P^H adjusted to 6.5 with 1M Potassium hydroxide at a flow rate of 1ml/min. The detection was made at 293 nm. Calibration curve was linear over the concentration range of 10-60 µg/ml of Balofloxacin. The propose method was validated as per the ICH guidelines. The method was accurate, precise, specific and rapid found to be suitable for the quantitative analysis of the drug and Pharmaceutical dosage form.
Key Words: Buffer, Acetonitrile, Balofloxacin, Tablets, Zodiac C_{18} column, RP-HPLC.

Introduction

Balofloxacin 1-cyclopropyl-6-fluro-8-methoxy-7-(3-methylaminopiperidin-1-yl)-4-oxoquinoline-3-carboxylic acid, is a broad spectrum fluorinated quinolone antibacterial. It exhibits excellent antibacterial activity against gram-positive bacteria such as multiple-drug-resistant staphylococci and pneumococci. It acts by binding to and inhibiting topoisomerase II (DNA-gyrase) and topoisomerase IV enzymes, which are responsible for the coiling and uncoiling of DNA, which is needed for bacterial cell repair and replication. It is not official in any of the Pharmacopoeia. A Literature survey reveals that various analytical methods, such as UV method (Punam M.Thumar, *et al*.2011[(1)]),Simultaneous HPLC method(H.garcia ovando, *et al*,2004 [(2)]), HPLC- electro spray ionization(ESI)–MS(Bian Z, *et al*,2007[(3)]), Human plasma by RP-HPLC(Song Qin-xin, *et al*,2008[(4)]), Biological fluids by HPLC(Nakagawa T , *et al* ,1995, Carlucci G,1998 [(5,7)]),Body fluids by HPLC(Kudo M, *et al* ,2001 [(6)]),biological matrices by liquid chromatography (Sousa J, *et al* , 2012 [(8)]).The present investigation by the author describes a rapid, accurate, precise and Specific, RP–HPLC method for the determination of Balofloxacin from bulk and pharmaceutical dosage form.

Experimental

Chromatographic Conditions: The determination was carried out on waters HPLC model 2695 equipped with UV Visible detector using data handling system-waters alliance empower two software. The column used in the development for determination is Zodiac C_{18} (150× 4.6mm, 5µ). The detector wavelength was set at 293 nm. A flow rate of 1ml/min was used for the determination of Balofloxacin. The mobile phase composition was 0.01 M Potassium dihydrogen orthophosphate: Acetonitrile in the ratio of 40:60 (v/v) and P^H adjusted to 6.5 with 1M Potassium hydroxide. The samples and standards were dissolved in the mobile phase and 20µl samples were injected into the HPLC system at the column temperature of 30ºC. The column was equilibrated with the mobile phase for at least 30 min prior to the injection of the drug solution

Chemicals: The reference sample and branded formulation was supplied by Bio Leo Analytical labs, Hyderabad, India.HPLC grade acetonitrile, water were purchased from E.Merck,Mumbai, India, and Potassium dihydrogen phosphate AR grade purchased from SD Fine Chem,Mumbai, India.

Preparation of mobile phase: Accurately 1.36g of potassium dihydrogen phosphate was weighed out and dissolved in 1000ml of water. The solution was filtered through 0.45µ membrane filter and was degassed. A freshly prepared buffer solution: Acetonitrile in a ratio of (40:60 V/V) was filtered through 0.05µ membrane filter and sonicated by using Power Sonicator (model no: 405, Hwashin Technology, Korea) before use. The flow rate of the mobile phase was maintained at 1ml/min. The column temperature was maintained at 30ºC and the detection of the drug was carried out at 293nm.

Preparation of Stock solution: About 40mg of Balofloxacin was weighed accurately and transferred into 100 ml volumetric flask, added 60 ml of diluent and the solution was sonicated to dissolve and dilute to the volume with mobile phase.

Linearity and Calibration Curve

Linearity of the peak area response was determined by taking aliquots ranging from 2.5-15 ml of the above stock solutions into different 100 ml volumetric flasks and diluting up to the mark with the mobile phase. 20µL quantity of each solution was injected each time in to the column at a flow rate 1ml/min.The drug in the elutes was monitored at 293 nm and the corresponding chromatograms were obtained. A linear relationship in the range was found to the 10-60µg/ml for Balofloxacin. Form these chromatograms the peak area were calculated and a plot of concentration over the peak area was constructed. The regression of the plot was completed by least squares regression method.

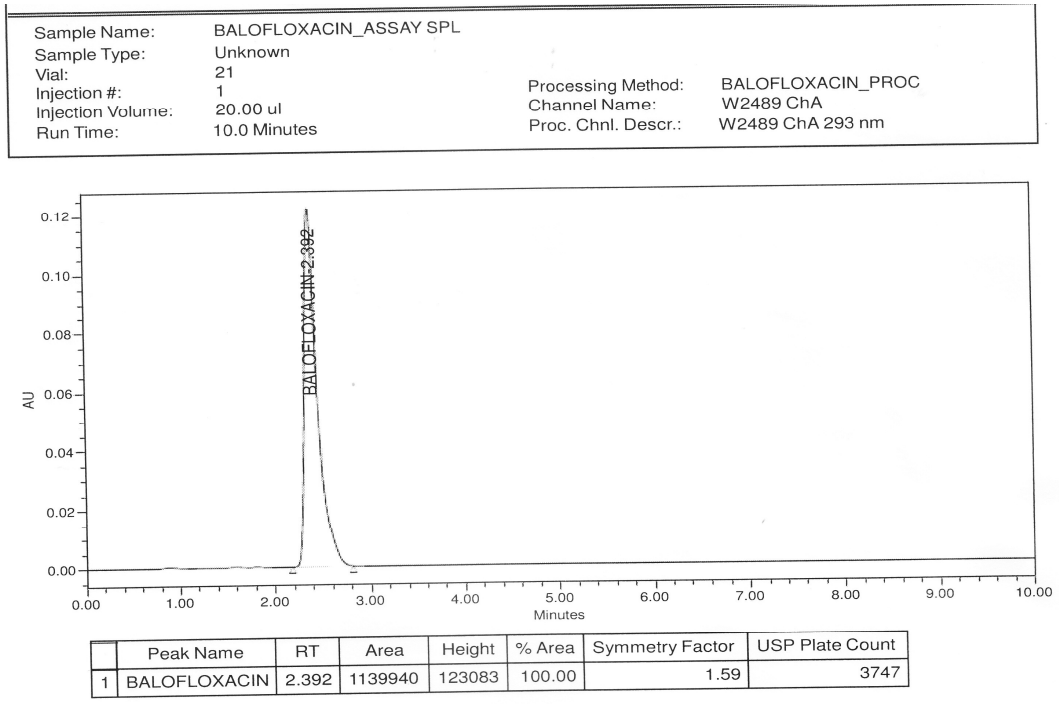
Preparation of Sample solution: Twenty tablets of Balofloxacin were weighed and powdered uniformly in a mortar. An accurately weighed portion powder equivalent to 40mg was transferred into 100 ml volumetric flask. The contents of the flask were sonicated for about 15 min for complete solubility of the drug and the volume was made up to 100ml with mobile phase. Then the mixture was filtered through a 0.45µ membrane filter. From the above solution 10ml aliquot was taken into a separate 100ml volumetric flask and diluted up to the volume with the mobile phase and mixed well. The above solution (20µL) was then injected Six times into the column and peak areas were measured and the quantitation was carried out by using calibration graph. A representative chromatogram for the separation of Balofloxacin is given in Figure 1.

Results and Discussion

In order to achieve optimum separation of the component peaks, mixtures of buffer: Acetonitrile in different combinations were tested as mobile phase on a Zodiac C_{18} column (150 mm x 4.6 mm;5µ) stationary phase. The mobile phase composition of 0.01 M Potassium dihydrogen orthophosphate: Acetonitrile in the ratio of 40:60 (v/v) and P^H adjusted to 6.5 with 1M Potassium hydroxide was selected as the chromatographic peaks were well defined and resolved with no tailing. The retention time obtained for Balofloxacin was 2.392 min. A good linear relationship (r^2 = 0.9999) was observed in the range of 10-60µg/ml for Balofloxacin. The lowest value of LOD and LOQ as obtained by the proposed method indicates the sensitivity of the method. The system suitability results and the regression characteristics are given in table 1. The drug content in tablets was quantified using the proposed analytical method are given in table 2.High recovery values obtained from the different dosage

form by the proposed method indicates the method is accurate.The deliberate changes in the method have not much affected the peak tailing, Theoretical plates and the percent assay indicates the robustness of the method. The robustness study results are presented in table 3. The system precision was established by six replicate injections of the standard solution containing analytes of interest. The value of relative standard deviation was found to be 0.31 indicating the injection repeatability of the method. The method precision was established by carrying out the analyte six times using the proposed method. The relative standard deviation was found to be 0.65 indicating the injection repeatability of the method. The specificity of the HPLC method was determined by forced degradation studies as per ICH guidelines. The results of specificity data for degradation study are given in table 4 and figures 2-5.

Figure 1: Chromatogram of Balofloxacin



	Peak Name	RT	Area	Height	% Area	Symmetry Factor	USP Plate Count
1	BALOFLOXACIN	2.392	1139940	123083	100.00	1.59	3747

Table 1: Optical and system suitability parameters

Parameter	**Balofloxacin**
Linearity range	10-60 µg/ml
Regression equation	
Slope	28778
Intercept	1500.6
Coefficient of correlation	0.9999
Limit of detection (LOD)	0.85
Limit of quantitation (LOQ)	2.58
System suitability	
Tailing factor	1.59
No. of theoretical plates	3844

Table 2:Analysis of Pharmaceutical Formulations

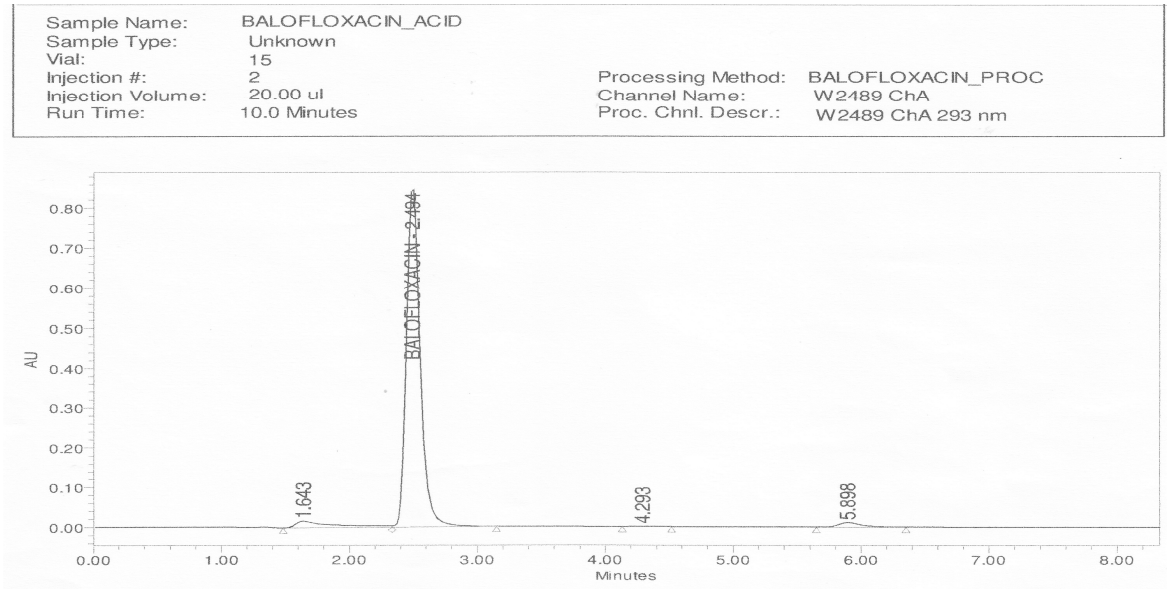
Drug	Percentage assay	System precision[a]	Method precision[a]	Percentage recovery[b]
Balofloxacin	99.93	0.31	0.65	98.86

[a] % RSD of Assay (N=6)
[b] Each value is a mean of three determinations at three different levels

Table 3: Robustness

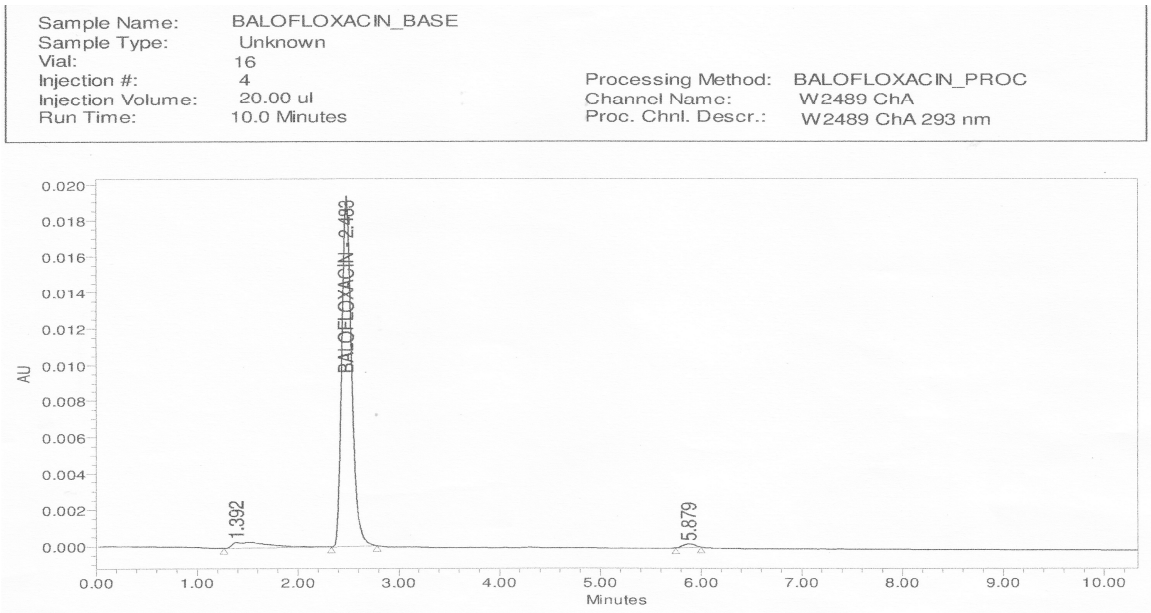
Variations	Chromatographic parameters				
	Retention time	Area	Height	Theoretical plates	Asymmetry
Change in Buffer at ± 5					
1.Buffer at 35:65	3.206	1106338	102338	3377	1.67
2.Buffer at 45:55	1.910	1155491	141920	3446	1.48
Change in flow rate at ±0.2ml/min					
1.flow rate at 0.8ml/min	2.613	1253175	126542	3892	1.62
2.flow rate at 1.2ml/min	2.195	1042648	118491	3638	1.55

Figure 2: Acid Degradation Chromatogram of Balofloxacin



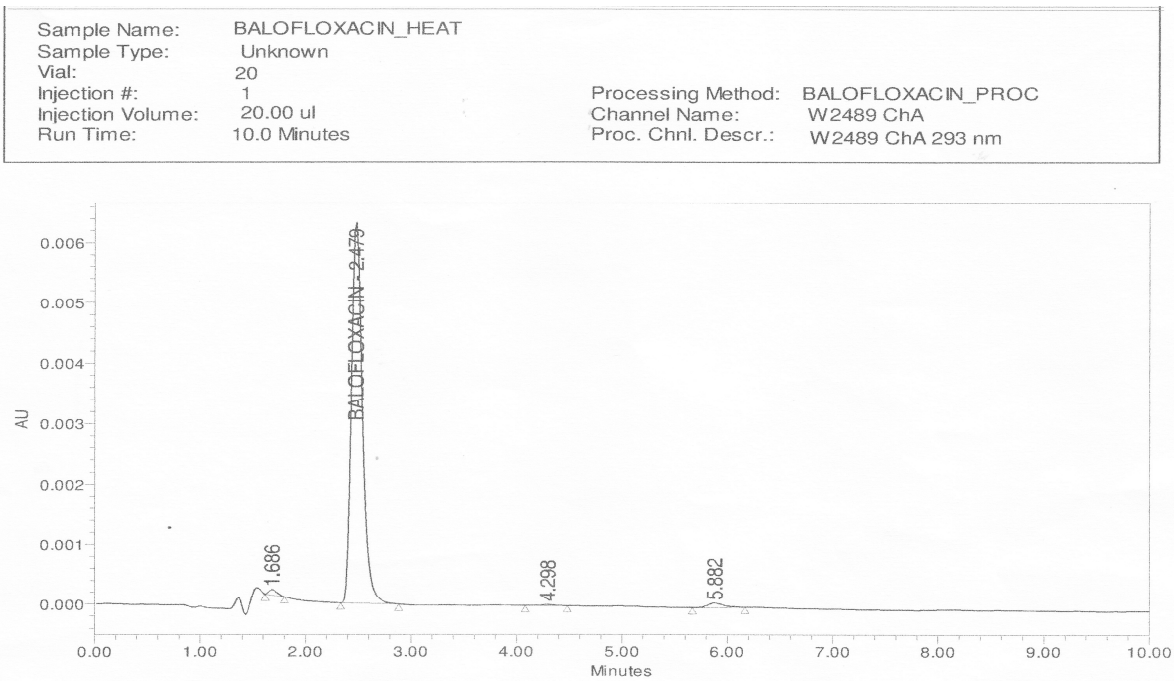
	Peak Name	RT	Area	Height	% Area	Resolution	USP Plate Count	Symmetry Factor
1		1.643	331850	16314	4.81		230.97	
2	BALOFLOXACIN	2.494	6415624	850282	93.03	3.03	2666.94	1.26
3		4.293	10270	1010	0.15	7.81	3942.65	1.33
4		5.898	138894	11301	2.01	5.58	5599.90	1.47

Figure 3: Base Degradation Chromatogram of Balofloxacin



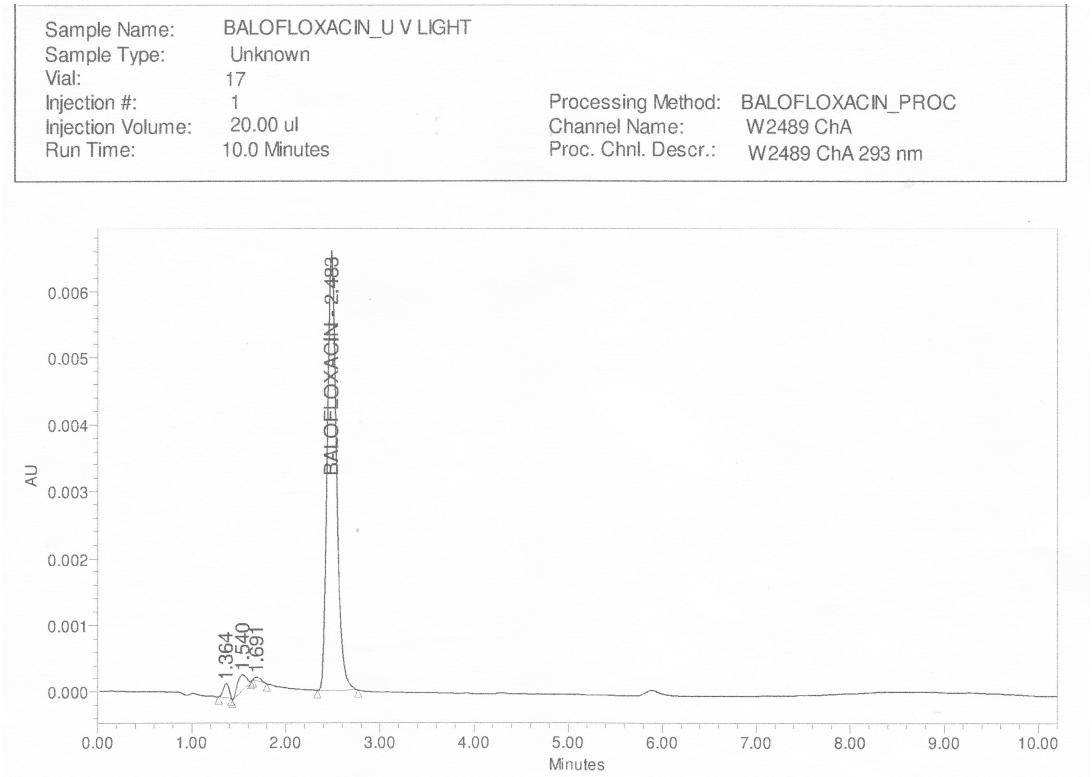
	Peak Name	RT	Area	Height	% Area	Resolution	USP Plate Count	Symmetry Factor
1		1.392	8025	324	5.85		79.39	4.22
2	BALOFLOXACIN	2.483	127656	19237	93.01	2.72	3378.33	1.22
3		5.879	1574	194	1.15	17.04	10604.00	0.98

Figure 4: Thermal Degradation Chromatogram of Balofloxacin



	Peak Name	RT	Area	Height	% Area	Resolution	USP Plate Count	Symmetry Factor
1		1.686	495	94	1.07		2216.06	1.36
2	BALOFLOXACIN	2.479	44980	6321	96.98	4.89	2933.81	1.32
3		4.298	151	15	0.32	8.32	4218.20	0.95
4		5.882	757	74	1.63	6.10	7659.25	1.19

Figure 5: Photolytic Degradation Chromatogram of Balofloxacin



	Peak Name	RT	Area	Height	% Area	Resolution	USP Plate Count	Symmetry Factor
1		1.364	843	209	1.79		2372.20	0.87
2		1.540	1642	245	3.48	1.19	1187.05	0.85
3		1.691	307	67	0.65	0.95	2999.08	1.77
4	BALOFLOXACIN	2.483	44408	6558	94.08	5.21	3239.09	1.25

Table 4: Forced Degradation

Condition	Time (hours)	Retention time (min)	Retention time of additional degradation peak (min)	% Degradation	% of Active drug Present after Degradation
Acid Degradation	06	2.494	1.643 4.293 5.898	4.81 0.15 2.01	93.03
Alkaline Degradation	06	2.483	1.392 5.879	5.85 1.15	93.01
Thermal Degradation	08	2.479	1.686 4.298 5.882	1.07 0.32 1.63	96.98
Photo Degradation	8	2.483	1.364 1.540 1.691	1.79 3.48 0.65	94.08

Conclusion

Hence it can be concluded that the proposed HPLC method is sensitive and reproducible for the analysis of Balofloxacin in pharmaceutical dosage form with short analysis time of 5 min.

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