CODEN (USA): IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

Available online at: http://www.iajps.com *Research Article*

STABILITY INDICATING RP-HPLC ASSAY METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF BALOFLOXACIN IN TABLETS

G. Bhavani, S.S. Hussain, S. Laxmi Prasanna, A. Ashok Kumar*
Department of Pharmaceutical Analysis and Quality Assurance, Vijaya College of Pharmacy, Munaganur (village), Hayathnagar (Mandal), Hyderabad – 501511, India.

Abstract:
The article aims at developing a rapid, sensitive, accurate, precise and linear stability indicating Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) assay method and validate as per ICH guidelines for the estimation of Balofloxacin in tablets. The optimized method employs a reverse phase column, Phenomenex Kinetex C18 (250X4.6mm;5µ), a mobile phase of Potassium dihydrogen phosphate buffer (pH 2.5):acetonitrile in the proportion of 50:50 v/v, flow rate of 0.6ml/min and a detection wavelength of 220 nm using a UV detector. Optimized method separated all the forced degradant impurities from the drug peak. Linearity of the method was 15-45µg/ml. Intra day and Inter day precision were exemplified by relative standard deviation of 1.09 and 1.17% respectively. Percentage mean recovery was found to be in the range of 90-110, during accuracy studies. The limit of detection (LOD) and limit of quantitiation (LOQ) was found to be 247.9ng/ml and 751.2ng/ml respectively.

Keywords: *Balofloxacin, stability indicating HPLC, validation.*

***Corresponding author:**
A. Ashok Kumar,
Professor and HOD,
Department of Pharmaceutical Analysis and Quality Assurance,
Vijaya College of Pharmacy, Munaganur (village),
Hayathnagar (Mandal), Hyderabad – 501511, India.
Email: ashok576@gmail.com

QR code

Please cite this article in press as Ashok Kumar et al , **Stability Indicating RP-HPLC Assay Method Development and Validation for the Estimation of Balofloxacin in Tablets ,** *Indo Am. J. Pharm. Sci, 2015;2(10).*

INTRODUCTION

Balofloxacin,(**Figure 1**) is a fourth generation Fluoroquinolone antibiotic used as a broad spectrum antibacterial drug exhibiting activity against Gram negative bacterium and anaerobe specially against Gram positive bacterium such as MRSA, Streptococcus pneumonia, Enterococcus faecalis. Balofloxacin chemically is 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylamino piperidin-1-yl)-4-oxoquinoline-3-carboxylic acid. Balofloxacin has an empirical formula of $C_{23}H_{26}N_2O_2.C_4H_6O_4$ and a molecular weight of 480.55. Mechanism of action includes inhibiting and binding with Topoisomerase II (DNA Gyrase) and Topoisomerase IV enzymes which are responsible for coiling and uncoiling of DNA needed for bacterial cell repair and replication [1-6]. Balofloxacin is prescribed for various infectious diseases such as opthalmitis, sinusitis, chronic bronchitis, acute exacerbation, community-acquired pneumonia, skin infections, urinary tract infections [7].

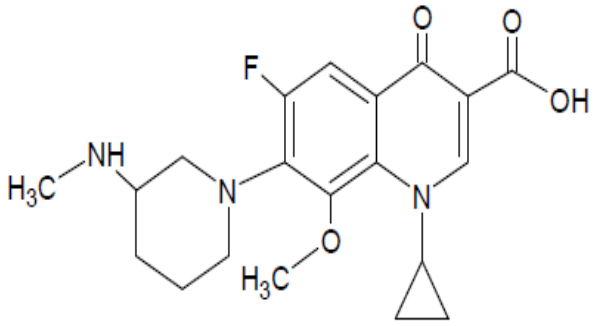

Fig. 1: Structure of Balofloxacin

Literature survey reveals few chromatographic methods [3,8-10] and few spectrophotometric methods [6,11-13] for the analysis of Balofloxacin in pharmaceutical dosage forms and few bioanalytical methods for the analysis of Balofloxacin in human plasma [14-16] and in urine [17]. We here report a totally new, rapid, simple, accurate, precise and linear stability indicating RP-HPLC isocratic assay method for the determination of Balofloxacin in tablets and validate the developed method as per ICH guidelines.

MATERIALS AND METHODS

Chemicals and Reagents

Analytically pure sample of Balofloxacin with purities greater than 99% was obtained as gift sample from Chandra labs, Hyderabad, India and tablet formulation [BALOFORCE] was procured from MEDPLUS Pharmacy, Hyderabad, India with labelled amount 100mg of Balofloxacin. Acetonitrile (HPLC grade), water (HPLC grade), Potassium dihydrogen phosphate (AR Grade) and ortho phosphoric acid (AR Grade) were obtained from SD Fine chemicals (Hyderabad, India), 0.45µm and 0.22 µm Nylon membrane filters were obtained from Spincotech Private Limited, Hyderabad, India.

Instrument

HPLC analysis was performed on Shimadzu Prominence Liquid Chromatograph comprising a LC-20AT pump, Shimadzu SPD-20A Prominence UV-VISIBLE detector and a reverse phase C18 column, Phenomenex Kinetex Make (250X4.6 mm; 5µ). A manually operating Rheodyne injector with 20 µL sample loop was equipped with the HPLC system. The HPLC system was controlled with "Spinchrom" software. An electronic analytical weighing balance (0.1mg sensitivity, Shimadzu AY 220), digital pH meter (DELUX model 101), a sonicator (sonica, model 2200 MH) and UV-Visible Spectrophotometer (Shimadzu UV-1800 series, software-UV probe version 2.42) were used in this study.

Method

Selection of Wavelength

Forced degradation samples, standard and blanks along with controls were injected into HPLC at various wavelengths *viz*. 220nm, 254nm, 280nm and 315nm. Significant impurities and majority of impurities along with the drug were detected at 220nm and hence was chosen as suitable wavelength.

Chromatographic Conditions

The optimized method employs a reverse phase column, Phenomenex Kinetex C18 (250X4.6mm;5µ), a mobile phase of Potassiumdihydrogen phosphate buffer (pH 2.5): acetonitrile in the proportion of 50:50 v/v, flow rate of 0.6ml/min and a detection wavelength of 220 nm using a UV detector.

Buffer Preparation

The buffer solution was prepared by weighing 2.736g of potassium dihydrogen orthophosphate (KH_2PO4) and transferring to 1000 ml of HPLC grade water to get 20 mM buffer strength, which was adjusted to pH 2.5 using 30%v/v ortho phosphoric acid. Later the buffer was filtered through 0.45 µm nylon membrane filter.

Mobile Phase Preparation

The mobile phase was prepared by mixing acetonitrile and buffer in the ratio of 50:50 v/v and later it was sonicated for 10 minutes for the removal of air bubbles.

Diluent

Mobile phase was used as a diluent.

Preparation of Stock and Working Standard Solution

10mg of Balofloxacin was accurately weighed and taken in 100 ml clean and dry volumetric flask containing 50 ml of diluents and then sonicated for 2 minutes to dissolve. Later the solution was made up to the mark using the mobile phase. This is considered as stock standard solution (100µg/ml).

From the stock solution, 3ml was pipetted out and to 10ml using the diluent to get a concentration of 30µg/ml, treated as 100% target concentration.

Preparation of Stock and Working Sample Solution

Ten tablets were weighed separately. The tablet powder weight equivalent to 10mg of Balofloxacin was weighed from the ten tablets grinded in a pestle and mortar, transferred to a 100 ml volumetric flask containing 70 ml diluent and then stirred for 5minutes, followed by filtration through 0.45µ nylon membrane filter and later made up to the mark to get sample stock solution of 100µg/ml. 3ml of the above stock solution was pipetted out and made up to 10 ml to get secondary working sample solution of 30µg/ml, equivalent to a concentration of working standard..

RESULTS AND DISCUSSION

Method Development

RP-HPLC isocratic stability indicating assay method was developed keeping in mind the system suitability parameters i.e. Asymmetric factor (A), number of theoretical plates (N), runtime, separation of drug peak from the forced degradants, detection of drug peak along with significant impurities and majority of impurities. In order to test the applicability of the developed method to a commercial formulation, BALOFORCE was chromatographed at working concentration (30µg/ml) and it is shown in **Figure 2.** The sample peak was identified by comparing the retention time with the standard drug. System suitability parameters were within the acceptance limits, ideal for the chromatographed sample. Integration of separated peak area was done and drug concentration was determined by using the peak area concentration relationship obtained in the standardization step. The protocol affords reproducible assay of the drug in the sample ranging between 90 and 110%, which is the standard level in any pharmaceutical quality control.

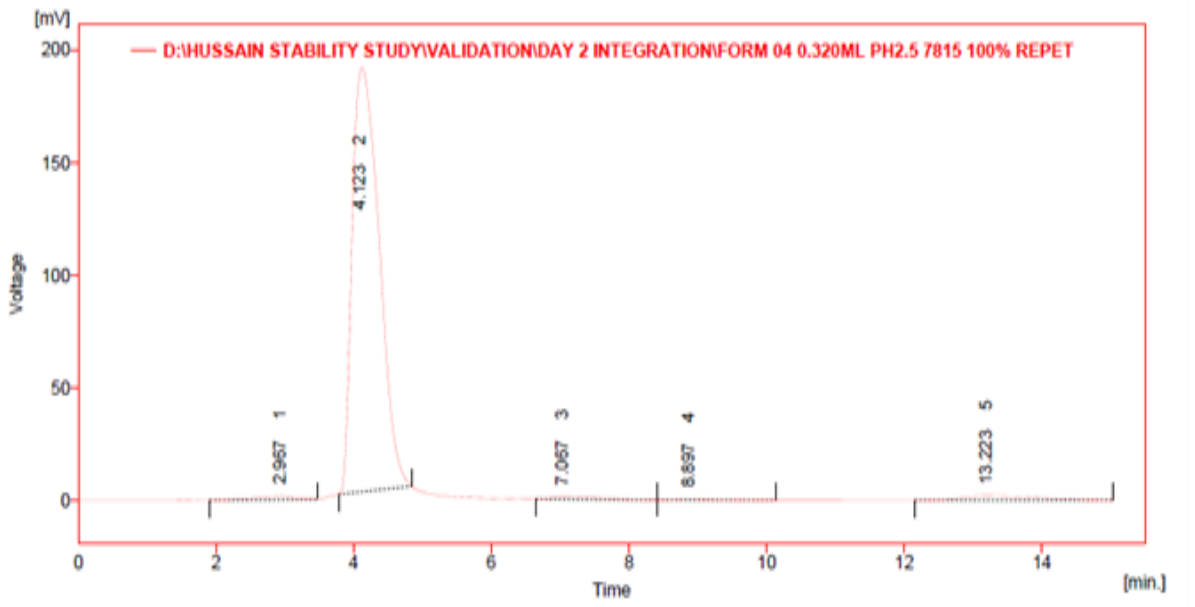

Fig.2: Typical chromatogram of the control formulation.

Method Validation

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. RP-HPLC method developed was validated according to International Conference on Harmonization (ICH) guidelines [18] for validation of analytical procedures. The method was validated for the parameters like system suitability, specificity, linearity, accuracy, precision, and sensitivity.

Specificity

Blank, standard drug solution and sample chromatogram revealed that the peaks obtained in the standard solution and sample solution at working concentrations are only because of the drug as blank had no peak at the retention time of Dabigatran etexilate mesylate. Accordingly it can be concluded that, the method developed is said to be specific.

Precision

System Precision

Six replicate injections of the standard solution at working concentration showed % RSD (Relative Standard Deviation) less than 2 concerning peak area for the drug, which indicates the acceptable reproducibility and thereby the precision of the system. System precision results are tabulated in **Table 1**.

Method Precision

Method precision was determined by performing assay of sample under the tests of repeatability at working concentration.

Repeatability (Intra day precision)

Six consecutive injections of the sample from the same homogeneous mixture at working concentration showed % RSD less than 2 concerning % assay for the drug which indicate that the method developed is method precise by the test of repeatability and hence can be understood that the method gives consistently reproducible results (**Table 2**).

Table 1: System Precision Results.

n	Pea k area
1	3623.034
2	3659.195
3	3666.017
4	3661.067
5	3666.669
6	3668.205
Average	3657.3645
STDEV	17.17322558
%RSD	0.469551929

Table 2: Intraday Precision Results.

n	Sample area	% Assay
1	4080.214063	105.9835
2	4108.234711	106.7113
3	4171.046424	108.3429
4	4194.28161	108.9464
5	4231.821983	109.9215
6	4157.119758	107.9811
Average	4172.500897	108.3807
STDEV	45.72261001	1.187644
%RSD	1.095808273	1.095808

Intermediate Precision (Ruggedness / Inter day precision)

Six consecutive injections of the sample solution from the same homogeneous mixture at working concentration on a different day by a different analyst, showed % RSD less than 2 for % assay for the drug within and between days, which indicate the method developed is inter day precise / rugged (**Table 3)**.

Table 3: Inter day Precision Results.

n	Sample area	% Assay
1	4786.974059	107.6204
2	4812.921983	108.2037
3	4684.918627	105.326
4	4832.702017	108.6484
5	4832.710246	108.6486
6	4765.158786	107.1299
Average	4785.89762	107.5962
STDEV	56.107762	1.26141
%RSD	1.172356086	1.172356

Linearity

Standard solutions of Balofloxacin at different concentrations level (50%, 75%, 100%, 125% and 150%) were prepared. Calibration curve was constructed by plotting the concentration level of drug versus corresponding peak area. The results show an excellent linear correlation between peak area and concentration level of drug within the concentration range (15-45µg/ml) for the drug and the results are given in **Table 4** and **Figure 3.** The correlation coefficient of Balofloxacin is 0.9958 and hence the method is said to be linear in the range of 15-45 µg/ml.

Table 4: Calibration Data of Balofloxacin.

n	% Level	Concentration (µg/ml)	Peak Area
1	50	15	2721.865
2	75	22.5	3867.632
3	100	30	5209.678
4	125	37.5	6317.537
5	150	45	7408.526
Regression coefficient		0.9985	
Regression equation		y=157.74x+371.18	

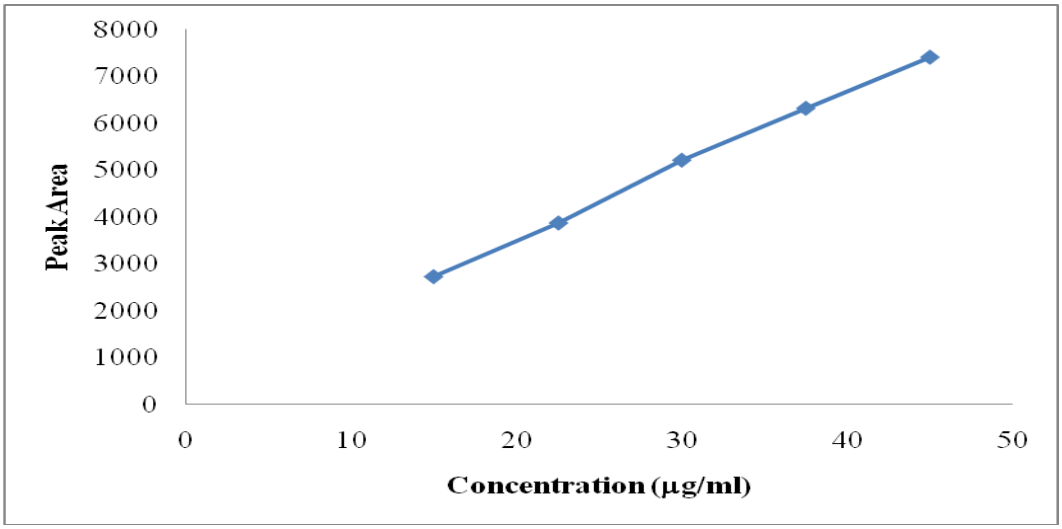

Fig.3: Linearity Graph of Balofloxacin

Accuracy

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample at three different levels (50-150%). At each level, three determinations were performed. Percent mean recovery was calculated as shown in **Table 5.** The accepted limits of recovery are 90%-110% for the process of determining recovery of the standard from the formulation at three different levels of 50%, 100% and 150%. All observed data are within the required range which indicates good recovery values and hence the accuracy of the method developed.

Table 5: Recovery Studies Results

%Level	Sample area	% Recovery	Average	%RSD
50-1	2349.2343	105.6306112	107.0338115	1.85545616
50-2	2361.111098	106.1646377		
50-3	2430.979402	109.3061854		
100-1	4786.974059	107.6203842	107.0500364	1.421110144
100-2	4812.921983	108.2037434		
100-3	4684.918627	105.3259817		
150-1	6273.146174	93.0813786	94.04883263	1.888094407
150-2	6265.431967	92.96691466		
150-3	6476.462787	96.09820463		

Sensitivity

The sensitivity of measurement of Balofloxacin by use of the proposed method was estimated in terms of the limit of quantitation (LOQ) and the limit of detection (LOD). LOQ and LOD were calculated by the use of the equations LOD = 3.3σ/S and LOQ = 10σ/S where σ is the standard deviation of response of calibration plot and S is the slope of the corresponding calibration plot. The limit of detection (LOD) and limit of quantitiation (LOQ) was found to be 247.9ng/ml and 751.2ng/ml respectively.

Forced Degradation Studies

Controls and forced degradation of blank, standard and sample were injected into HPLC system. Each and every forced degradation condition was optimized by changing the strength and volume of the reagent, temperature and time of exposure till there exist degradation significantly. **Figures 4 to 11** represent chromatograms of forced degradation samples under optimized conditions of acidic, basic, neutral, oxidation, uv, visible and dry heat. **Table 6** summarizes the optimized forced degradation conditions and %degradation observed under each condition along with impurities detected at 220nm with their percentages.

Table 6: Forced Degradation Studies of the Sample:

Optimized Degradation conditions	% Degradation	RT of impurities in min and (percentage)
Acidic (1ml of 1N HCl, 70-80°C, 3 hours)	71.15	0.747 (1.5%)
		7.723 (10.4%)
Basic (1ml of 1N NaOH, 70-80°C, 10min)	47.57	7.043 (29.5%)
Neutral (3ml water, 70-80°C for 3 hours and later kept overnight for 48 hours at rt)	65.79	1.123(19.1%)
		5.945(0.365%)
Oxidation (5ml of 5%H_2O_2 kept overnight at rt)	54.63	0.117(1.1%)
		3.27 (0.9%)
		6.327 (1.6%)
		14.637 (7.3%)
UV (Short for 7 days and later Long 7 days)	53.14	2.45(9.6%)
Light (15 days under sunlight)	65.15	-
Dry Heat (75-85°C,10 days)	54.81	-
Humidity (test performed for 20 days by placing formulation in dessicator filled with saturated potassium chloride solution)	84.13	-

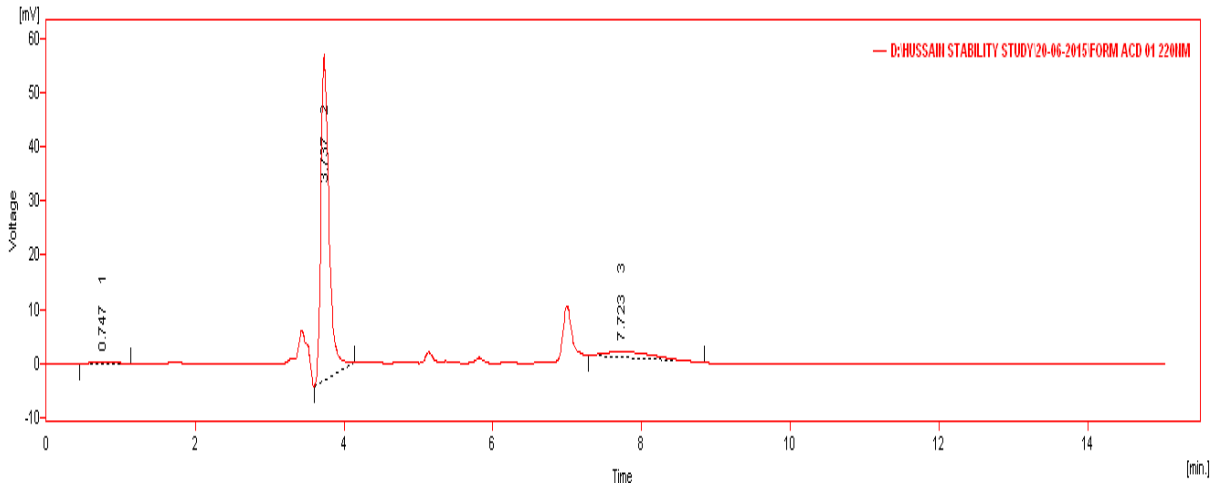

Fig. 4: Chromatogram of the Sample under Acidic Degradation

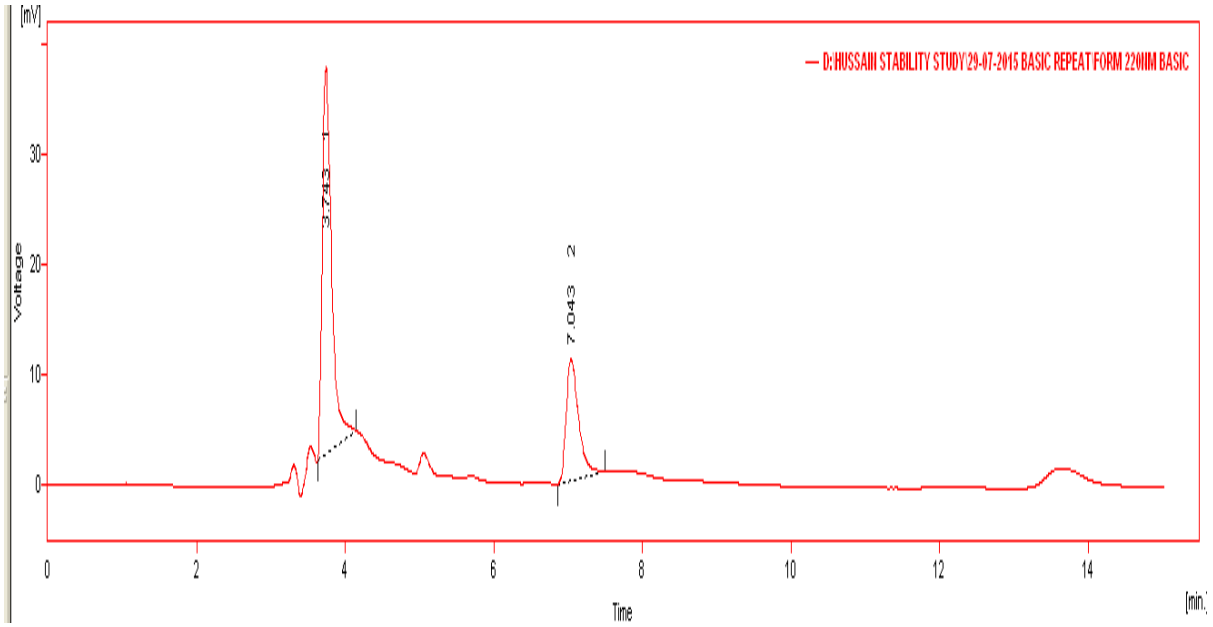

Fig. 5: Chromatogram of the Sample under Basic Degradation

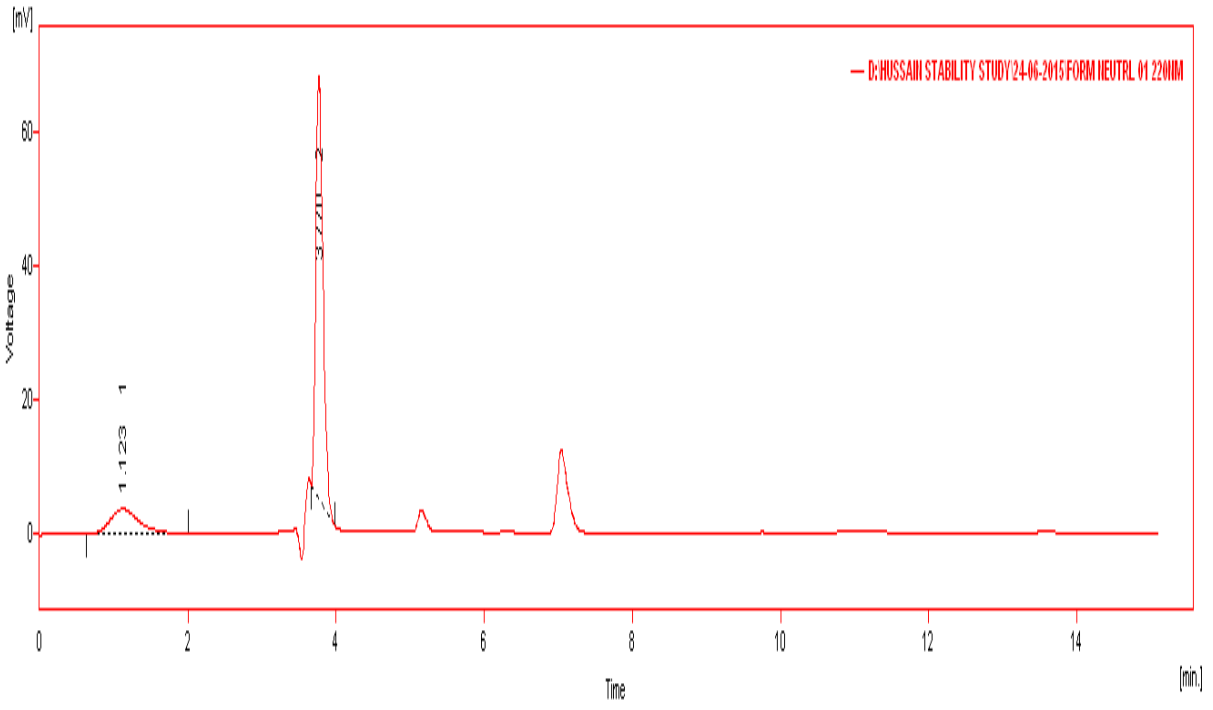

Fig. 6: Chromatogram of the Sample under Neutral Degradation

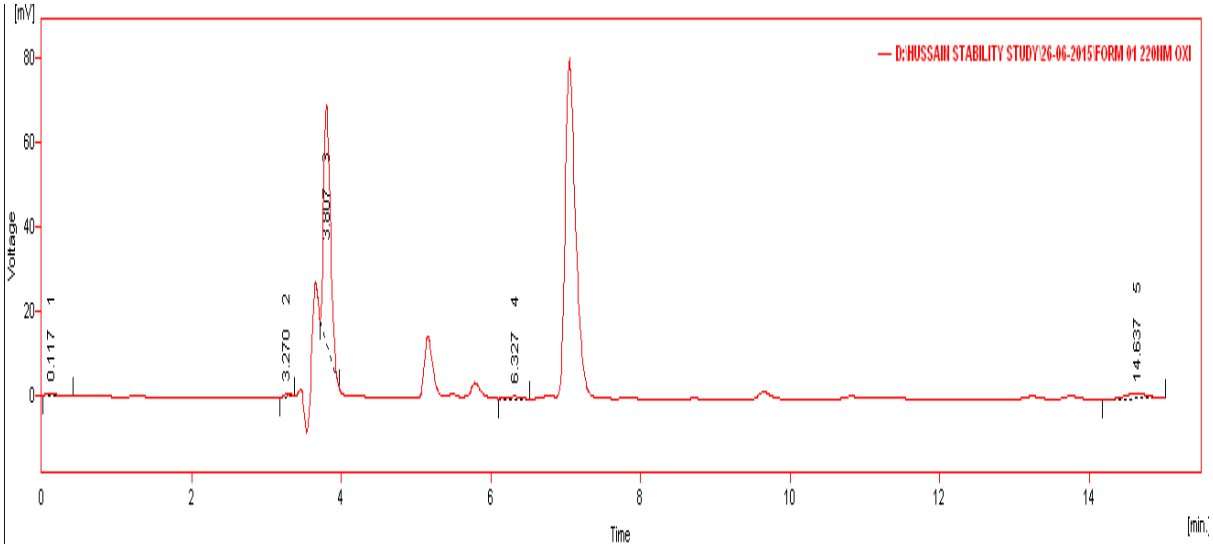

Fig. 7: Chromatogram of the Sample under Oxidative Degradation

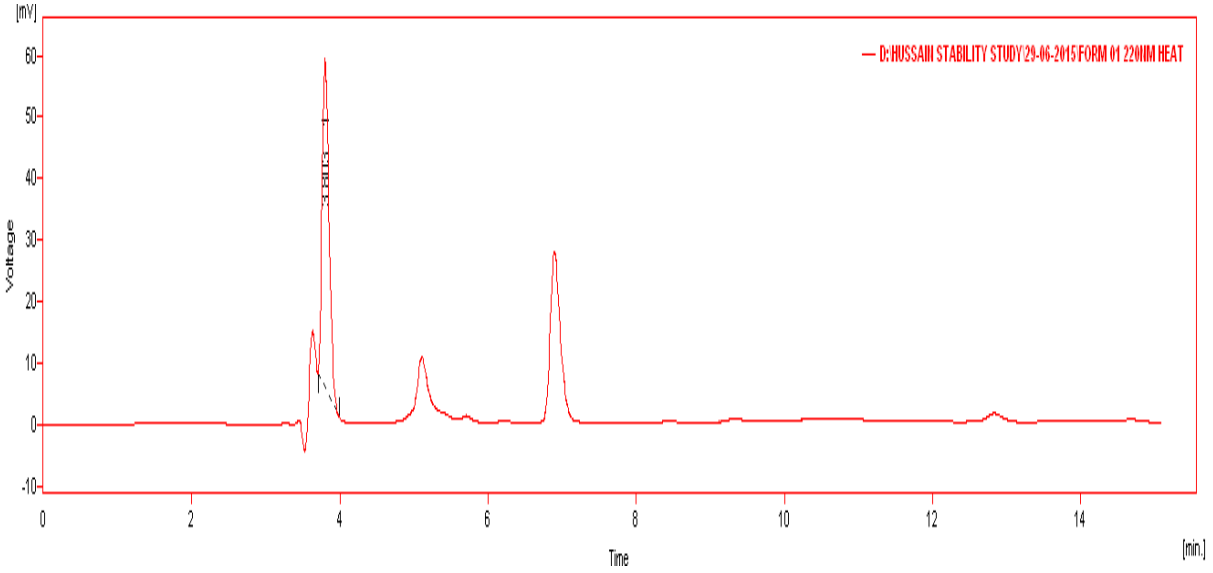

Fig. 8: Chromatogram of the Sample under Dry Heat Degradation

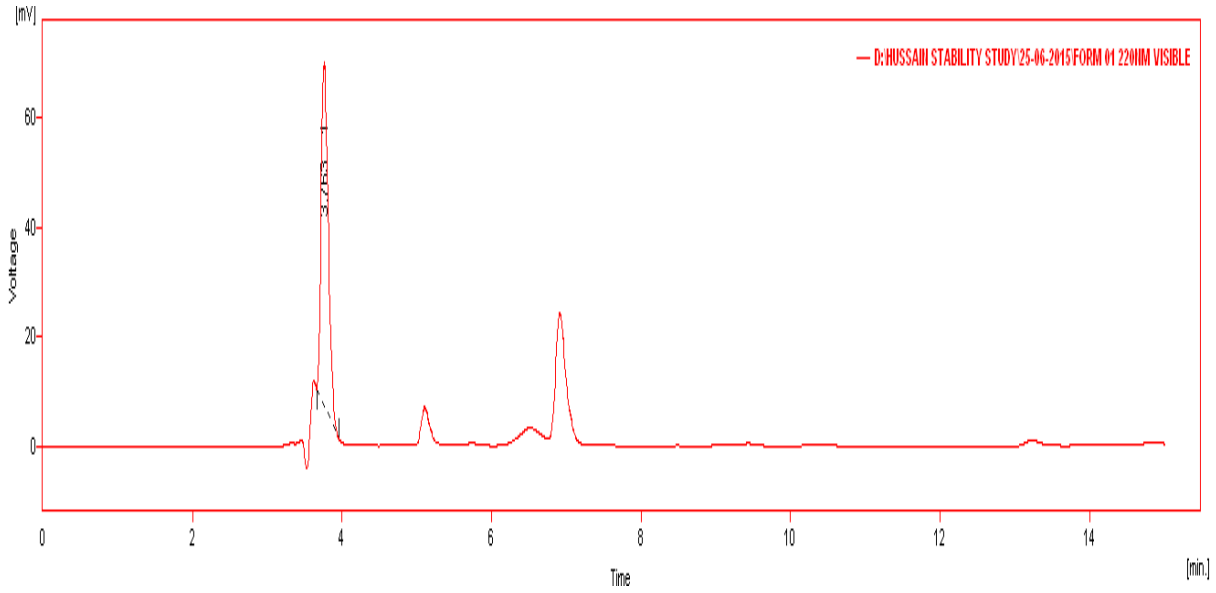

Fig. 9: Chromatogram of the Sample under Sunlight Degradation

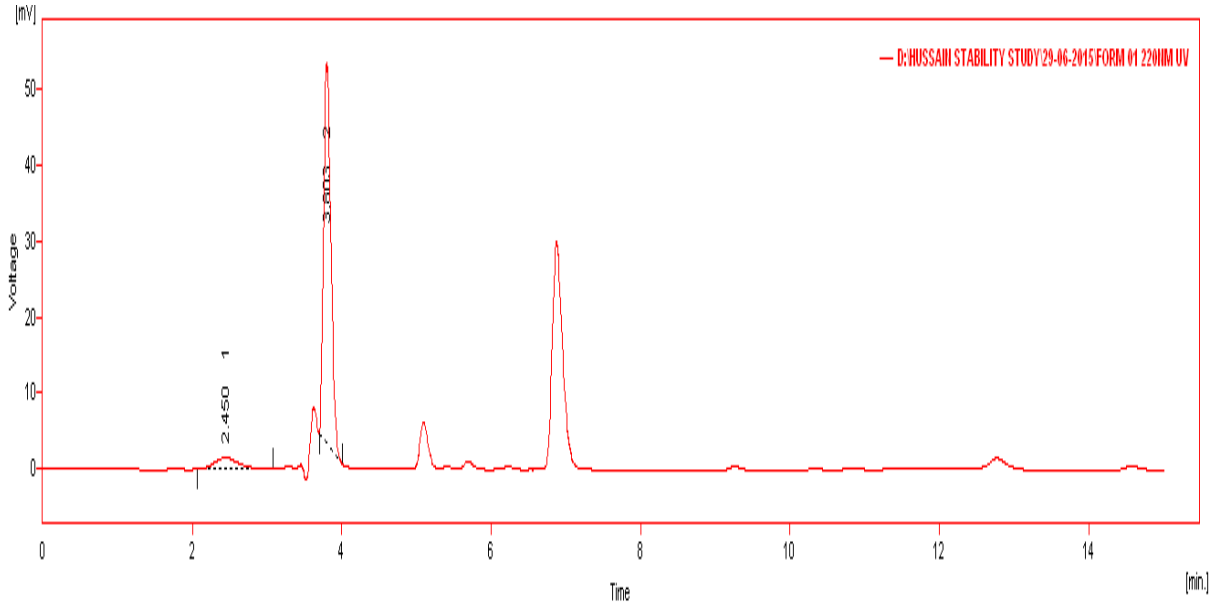

Fig. 10: Chromatogram of the Sample under UV Degradation

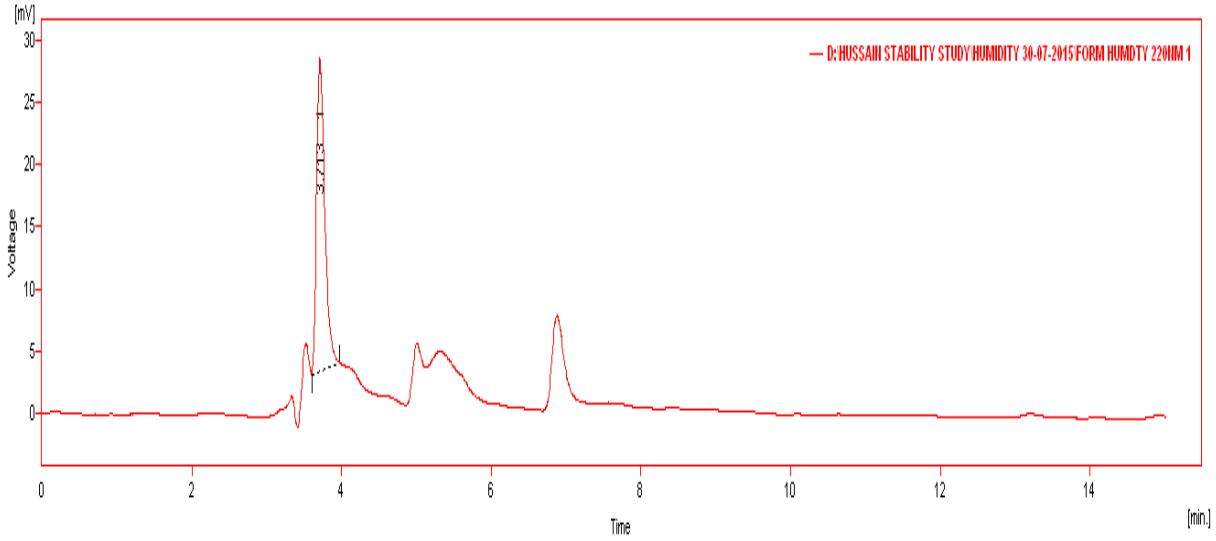

Fig. 11: Chromatogram of the Sample under Humidity Degradation

CONCLUSION

A reverse phase HPLC isocratic stability indicating assay method has been developed and validated as per ICH guidelines for the quantitative estimation of Balofloxacin in tablets. Forced degradants were separated from the drug peak using the optimized method. Intra day and Inter day precision were exemplified by relative standard deviation of 1.09 and 1.17% respectively. A good linear relationship was observed for the drug between concentration ranges of 15 and 45µg/ml. Accuracy studies revealed that mean recoveries were between 90 and 110%, an indicative of accurate method. The limit of detection (LOD) and limit of quantitiation (LOQ) was found to be 247.9ng/ml and 751.2ng/ml respectively. Accordingly it can be concluded that the developed reverse phase isocratic HPLC stability indicating assay method is sensitive, accurate, precise and linear and therefore the method can be used for the routine analysis of Balofloxacin in tablets.

ACKNOWLEDGEMENT

The authors thank the management of Vijaya college of pharmacy (VJYH), Hyderabad, for providing the necessary facilities to carry out of this research work. The authors are grateful to Chandra labs, Hyderabad for providing gift sample of Balofloxacin.

REFERENCES

1.Ross DL, Elkinton S, Riley CM. Physicochemical properties of the fluoroquinolone antimicrobials IV 1-octanol/water partition coefficients and their relationships to structure. Int. J. Pharm 1992;88:379-389.

2.Ashok Reddy S, Chandra Sekhar KB. Development and validation of analytical method for estimation of balofloxacin in bulk and Pharmaceutical dosage form. J of Global Trends in Pharm Sci 2012;3(2):647-655.

3.Ravisankar P, Devalarao G, Devadasu Ch, Gopal reddy P, Srinivasa babu P, Afzal basha SK, Sricharitha P. A novel validated RP-HPLC method for the determination of Balofloxacin in bulk and pharmaceutical dosage forms. International Journal of pharmacy and Industrial research 2013;8(2):127-136.

4.Ito T, Otsuki M, Nishino T. Invitro antibacterial activity of Q-35, a new fluoroquinolone. Antimicrob Agents Chemother 1992;36:1708-1714.

5. Gohara Y, Arai S, Akashi A, Kuwano K, Tseng CC, Matsubara S, Matumoto M, Furudera T. Invitro and Invivo activities of Q-35, a new fluoroquinolone, agaist Mycoplasma pneumonia. Antimicrob Agents Chemother 1993;37:1826-1830.

6. Seetharaman R, Lakshmi KS, Dinesh kumar B, Hariprasad G, Ravi KR. Determination of Balofloxacin in pharmaceutical formulation by Zero, First and Second order derivative spectrophotometric methods. International Journal of research in pharmaceutical sciences, 2011;2(3):438-443.

7.Alksne L. Balofloxacin choongwae. Curr Opin Investig Drugs 2003;4(2):224-229.

8. Nyola NK, Govindasamy J. Estimation of Balofloxacin in active pharmaceutical ingredients and pharmaceutical formulations by different methods. Novel Sci Int J of Pharm Sci 2012;1(7):425-429.

9.Naga Raju P; Devala Rao G; Rajendra Prasad Y.Development and validation of a Reverse Phase-HPLC Method for the determination of Balofloxacin in bulk and pharmaceutical dosage forms. Drug Invention Today 2012;4(12):655-658.

10.Thumar PM, Patel VB. Development and validation of analytical method for estimation of Balofloxacin in bulk and pharmaceutical dosage Form. Int J of Pharm Tech Res 2011;3(4):1938-1941.

11.Pai S, Nayak S. Development of a new spectroscopic method for determination of Balofloxacin in tablet dosage Form. Int J of Scientific Res 2013;2(7):76-77.
12.Pai SP. Spectrophotometric determination of Balofloxacin by ion-pair complexation reaction in the bulk and tablet dosage forms. Asian J of Pharm and Clinical Res 2013;1(1):22-24.
13.Reddy SA, Sekhar KC. Development and validation of analytical method for estimation of Balofloxacin in bulk and pharmaceutical dosage Form. J of Global Trends in Pharm Sci 2012;3(2):647-655.
14. Bian Z, Tian Y, Zhang Z, Xu F, Li J, Cao X. High Performance Liquid Chromatography–Electrospray Ionization Mass spectrometric determination of Balofloxacin in human plasma and its pharmacokinetics. J Chromatogr B Analyt Technol Biomed Life Sci 2007;850(1):68-73.
15.Wang D; Xie L; Wang J; Liu X. Determination of Balofloxacin in plasma by HPLC-Fluorescence and its pharmacokinetics in rats. Journal of China Pharmaceutical University 2004;35(2):160-163.
16.Chu ZJ, Dong YB, Yang FH, Wang L. Determination of the content of Balofloxacin in human plasma by RP-HPLC method and its application in therapeutic drug monitoring. J Pharm Care and Res 2008;8(3):223-225.
17. Sui Y;Guo T; Zhang J; Shi G; Tang Y; Zhao L. Determination of Balofloxacin in human urine by RP-HPLC with fluorescence detection. Journal of Shenyang Pharm University 2007;11:691-694.
18. International conference on harmonization of technical requirements for registration of pharmaceuticals for human use. Validation of analytical procedures: Text and methodology ICH Q2 (R1). 2005.