AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Development and Validation of UPLC Method for Estimation of Balofloxacin in Tablet Dosage Form

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ABSTRACT

A novel reverse phase Ultra performance liquid chromatographic technique was developed for the determination of balofloxacin in bulk and pharmaceutical dosage forms. The method was developed using waters Acquity BEH 50mm, 2.1mm, 2µm, C 18 column with mobile phase containing a gradient mixture of 0.1% phosphoric acid and acetonitrile. Detection was carried out at wavelength 295 nm. The retention time of balofloxacin was 0.89 min. The method showed good linearity in the range 0.5, 1, 1.5,2,3 µg/ml with correlation coefficient for balofloxacin. The proposed method has been validated as per ICH guidelines and successfully applied to the estimation of balofloxacin in their tablet dosage form.

Keywords: Balofloxacin, Ultra performance liquid chromatography, validation

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Received 18 August 2013, Accepted 24 August 2013

Please cite this article in press as: Malathi S. *et al* Development and Validation of UPLC Method for Estimation of Balofloxacin In Tablet Dosage Form. American Journal of PharmTech Research 2013.

INTRODUCTION

Balofloxacin chemically (1-cyclopropyl-6-fluoro-1, 4-dihydro -8-methoxy-7(3 methyl amino-piperidin-1-yl)-4-oxoquinolone-3-carboxylic acid(Figure.1). It has a broad anti bacterial Spectrum, ranging from gram positive bacteria to gram negative bacteria. In literature, various analytical methods, such as RP-HPLC, UV have been developed for determination of balofloxacin. Though high performance liquid chromatography (HPLC) is a well established reliable technique used in controlling the quality and consistency of active pharmaceutical ingredients (API) and dosage forms. It is often a slow technique because of the complexity of some of the samples, it could still be improved. Ultra performance liquid chromatography (UPLC) is a new category of separation technique based upon well established principles of liquid chromatography, which utilizes sub 2µm particles for stationary phase. Because of its speed and sensitivity, this technique is gaining considerable attention in recent years for pharmaceutical and biomedical analysis. In the present work, this technology has been applied to the method development and validation study of estimation of balofloxacin in bulk drug and dosage forms.

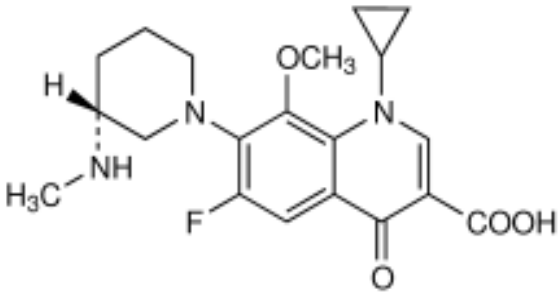

Figure 1: Structure of Balofloxacin

However there are no reports available on method development for balofloxacin API and dosage form. It is therefore necessary to develop a new method for quantitative estimation of balofloxacin.we intends to opt for a faster chromatographic technique UPLC for the said study. Hence a reproducible UPLC method was developed for the quantitative determination of balofloxacin.

MATERIALS AND METHODS:

Chemicals, reagents and drug formulations

Tablet formulation manufactured by hetero labs containing balofloxacin 100 mg was purchased from local market and were used for analysis. The acetonitrile used in the study were of UPLC grades were obtained from rankem Ltd. Water was prepared by using Millipore Milli Q plus water purification system.

Instrumentation

LC was carried out on waters Acquity UPLC with photodiode array detector. The output signal was monitored and processed using empowers software. The chromatographic column used Aquity UPLC BEH C18 50mm 2.1mm and 2µm particle size. The separation was achieved on a gradient method. The mobile phase A contains 0.1% phosphoric acid and mobile phase B contains acetonitrile in the ratio 90:10 v/v respectively. The flow rate of mobile phase was 0.7 ml/min. The UPLC gradient program was set as time (min)/%solution. 0.00/A:90 B:10,1.00/A:10 B:90,1.5/A:10 B:90,2.00/A:90 B:10,3.0/A:90 B:10..The column temperature was maintained at 35°c and the detector was monitored at a wavelength 295 nm. The injection volume was 2 µl.

Preparation of standard stock solution

A stock solution of balofloxacin was prepared by 10mg of drug was accurately weighed, transferred to 10 ml volumetric flask and dissolved in 10 ml of methanol and sonicated for 10 mins(1000 µg/ml).Further dilutions are made from stock to get required working standard concentrations. (0.5 µg to 3.0 µg/ml).

Development and optimization of chromatographic condition

The UPLC procedure was optimized with a view to develop an assay method for Balofloxacin. The standard stock solution was injected in UPLC. For UPLC method optimization different ratios of different mobile phases were tried in combination with different columns including water, phosphate buffers, methanol as the mobile phase and different columns with various dimensions. But it was found that none of these combinations worked. After many trials it was found that the chromatogram was achieved on a gradient method. (Figure2). The mobile phase A contains 0.1% phosphoric acid and mobile phase B contains Acetonitrile in the gradient mixture ratio respectively.

Optimized chromatographic conditions:

In the optimized chromatographic conditions, the column used was Acquity UPLC, BEH C-18, 50X2.1, 2 µ column. The mobile phase comprised of 0.1%phosphoric acid and acetonitrile in the ratio of 90:10 in gradient method. The flow rate was 0.7 ml/min and the detection wavelength was 295 nm. The injection volume was 2 µl and the temperature was 35°c.

RESULTS AND DISCUSSION

System suitability parameters

To ascertain resolution and reproducibility of proposed chromatographic system for estimation of balofloxacin in tablets, system suitability parameters like tailing factor(T), and column

efficiency (number of theoretical plates, N) were studied. Standard stock solution containing balofloxacin (100 µg/ml) was used for analysis. The filtrate (2µl) was injected into the column and chromatographed using optimized chromatographic conditions. The system suitability test was performed from six replicate injections of standard solution. The chromatogram was recorded at 295 nm.Typical chromatogram obtained, is given in system suitability parameters were calculated and are presented in Table.1.

Table 1: System Suitability Parameters

Sr.No	Parameter	Balofloxacin
1	Retention time (Rt)	0.89
2	Tailing factor (Tf)	1.4
3	No of theoretical plates (N)	8891

METHOD VALIDATION

Linearity:

The linearity studies showed that the drug had a linear response in the stated range of 0.5 to 3 µg/ml the co-efficient of correlation was 0.9999 for balofloxacin which indicate a good linearity. (Figure.3).

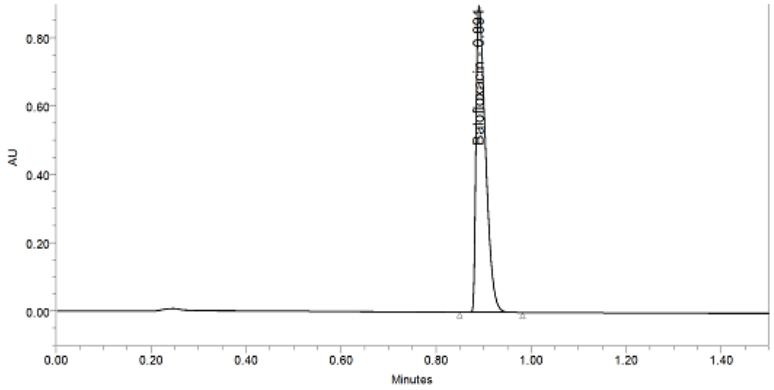

Figure 2: Chromatogram of Balofloxacin

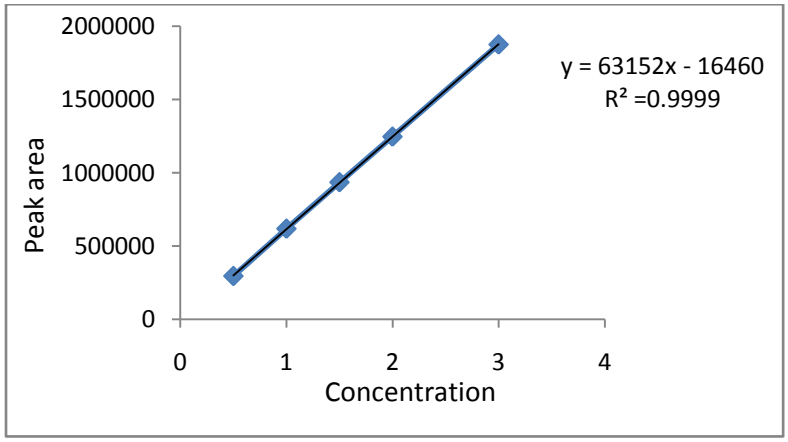

Figure 3: Calibration Curve of Balofloxacin

Precision:

The intra-day and the inter day precision results show that there is not much variation in the analysis results and the %RSD was always less than 1.00. (Ideally should be less than 2.0).The results of intra day and inter day precision are shown in Table.2.

Table 2: Precision

Intraday precision	% RSD	Inter day precision	%RSD
294488	0.1%	294488	0.1%
294510		294460	
294490		294427	

Accuracy:

The results of recovery studies of the marketed formulation are indicates that there is no interference in the analysis of the drug from the excipients in the tablet formulation.The results of recovery studies at various levels shows that the recovery is between 97.52-100.8 (ideally should be between 98-102%).Twenty tablets were weighed accurately, powdered and a quantity of tablet powder equivalent to 100 mg of balofloxacin was weighed and dissolved in the 100 ml of methanol with the aid of ultrasonication for 10 min and solution was filtered through whatmann paper no 41 into a 100ml volumetric flask. Further take 10ml of this solution to 100ml volumetric flask with methanol to get 100 µg/ml of solution. From this solution appropriate dilutions were made and injected in to the system to get the chromatogram.

The tablets, purchased from local market, were analyzed and the obtained results are compared to the label claim .From these data it can be concluded that the proposed method is suitable and specific for the estimation of balofloxacin in the tablet dosage form. There is no interference in the estimation of balofloxacin by the excipients in the tablet dosage form. In peak purity analysis with photo diode array detector, purity angle was always less than purity threshold for the analysis. The above discussion leads to a conclusion that the method can be easily applied to the estimation of balofloxacin as an industrially applicable method. The results of analysis of marketed formulation are shown in Table.3.

Table 3: Analysis of Formulation

Labeled amount (mg/tablet)	Calculated amount (mg/tablet)	% Label claim	%RSD*
100	98.62	98.62	0.3

* RSD of six observations

Robustness:

The robustness studies show that after deliberate changes in the various parameters there is not much change in the system suitability parameters.

CONCLUSION:

UPLC method was developed and validated as per ICH guidelines.The method is specific for estimation of balofloxacin in pharmaceutical dosage form. The method has linear response in stated range of 0.5- 3µg /ml of balofloxacin and is accurate and precise. The %RSD during precision was always less than 2 and recovery studies at various levels i.e. 50,100,120%. Robustness studies did not show any significant change in the various system suitability parameters nor were the assay values significantly changed by minute changes. Statistical analysis proves that the method is suitable for the analysis of balofloxacin as bulk drugs and in pharmaceutical formulations.

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