A NOVEL RAPID RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE ESTIMATION OF BALOFLOXACIN IN TABLETS

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ABSTRACT
Objective: To develop a rapid, accurate, precise and linear Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method and validate as per ICH guidelines for the quantitative estimation of Balofloxacin in tablets.

Methods: The optimized method uses a reverse phase column, Enable Make C18G (250 X 4.6 mm; 5μ), a mobile phase of triethylammonium phosphate buffer (pH 2.3): acetonitrile in the proportion of 20:80 v/v, flow rate of 1.0 ml/min and a detection wavelength of 280 nm using a UV detector.

Results: The developed method resulted in Balofloxacin eluting at 2.6 min. Balofloxacin exhibited linearity in the range 2.5-7.5 μg/ml. The precision is exemplified by relative standard deviation of 1.08%. Percentage Mean recovery was found to be in the range of 98-102, during accuracy studies.

Conclusion: A rapid, accurate, precise and linear isocratic RP-HPLC method was developed and validated for the quantitative estimation of Balofloxacin in tablets as per ICH guidelines and hence it can be used for routine analysis in various pharmaceutical industries.

Keywords: RP-HPLC, Balofloxacin, Method development, Validation.

INTRODUCTION
Balofloxacin (fig. 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_{2}O_{4}. C_{4}H_{6}O_{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 ophthalmitis, sinusitis, chronic bronchitis, acute exacerbation, community-acquired pneumonia, skin infections, urinary tract infections [7].

Fig. 1: Structure of Balofloxacin

Literature survey reveals chromatographic methods [3,8-10] and 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]. In this article, we report a totally new, simple, accurate and precise RP-HPLC isocratic method for the determination of assay 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 the gift sample from Chandna 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), Triethylamine (AR Grade) and ortho phosphoric acid (AR Grade) were obtained from SD Fine chemicals (Hyderabad, India), 0.45μm Nylon membrane filters were obtained from Spincotech Private Limited, Hyderabad, India.

Instrument
HPLC analysis was performed on Shimadzu Prominence Liquid Chromatography comprising a LC-20AD pump, Shimadzu SPD-20A Prominence UV-VISIBLE detector and a reverse phase C18 column, Enable Make C18G (250 X 4.6 mm; 5μ). A manually operating Rheodyne injector with 20 μl sample loop was equipped with the HPLC system. The HPLC system was controlled with "Labolutions lite" 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
Suitable wavelength for the HPLC analysis was determined by recording UV spectrum in the range of 200-400 nm for Balofloxacin. Suitable wavelength selected was 280 nm (fig. 2).

Fig. 2: UV spectrum of Balofloxacin
Chromatographic conditions

The developed method uses a reverse phase C18 column, Enable Make C18G (250 X 4.6 mm; 5 μ), mobile phase consisting of triethylammonium phosphate buffer (adjusted using 30% v/v of ortho phosphoric acid pH 2.3): acetonitrile in the proportion of 20:80 v/v. The mobile phase was set at a flow rate of 1.0 ml/min and the volume injected was 20μl for every injection. The detection wavelength was set to 200 nm.

Buffer preparation

The buffer solution was prepared by adding 5 ml of triethylamine to 1000 ml of HPLC grade water and later pH was adjusted to 2.3 using 30% v/v of ortho phosphoric acid in water. The buffer was then 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 80:20 v/v and later it was sonicated for 10 minutes for the removal of air bubbles.

Diluent

100% pure acetonitrile is 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 this stock solution, 0.5 ml was pipetted out and made up to 10 ml using the diluent to get a concentration of 5μg/ml, treated as 100% target concentration.

Preparation of stock and working sample solution

Ten tablets were weighed separately and the average weight was determined. The average weight was weighed from the ten tablets grinded in a pestle and mortar, transferred to a 100 ml volumetric flask containing 100 ml diluent and then stirred for 25 minutes followed by filtration through 0.45μ nylon membrane filter to get primary sample stock solution of 100μg/ml. From this stock solution, 0.5 ml was pipetted out and made up to 10 ml using the diluent to get a concentration of 5μg/ml, equivalent to a concentration of working standard of 5 μg/ml.

RESULTS AND DISCUSSION

Method development

A Reverse phase HPLC method was developed keeping in mind the system suitability parameters i.e. tailing factor (T), number of theoretical plates (N), runtime and the cost effectiveness. The optimized method developed resulted in the elution of Balofloxacin at 2.6 min. Fig. 3 and 4 represent chromatograms of blank solution and the standard solution (5μg/ml) respectively. The total run time is 5 minutes. System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (Rt), number of theoretical plates (N) and peak Asymmetric factor of the standard at the working concentration are given in table 1.

In order to test the applicability of the developed method to a commercial formulation, BALOFORCE was chromatographed at working concentration (5μg/ml) and it is shown in fig. 5. The sample peak was identified by comparing the retention time with the standard drug fig. 4. 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 98 and 102%, which is the standard level in any pharmaceutical quality control.

Specificity

Fig. 3-5 for blank, standard drug solution and sample chromatogram reveal that the peaks obtained in the standard solution and the sample solution at working concentrations are only because of the drugs as blank has no peak at the retention time of Balofloxacin. 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 the 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 2.

Table 1: System suitability studies results

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Balofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time (min)</td>
<td>2.6</td>
</tr>
<tr>
<td>Number Of Theoretical plates (N)</td>
<td>3453</td>
</tr>
<tr>
<td>Tailing factor (T)</td>
<td>1.6</td>
</tr>
</tbody>
</table>
Method precision

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

Repeatability (Intraday 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 3).

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 6. The accepted limits of recovery are 98% - 102% and all observed data are within the required range which indicates good recovery values and hence the accuracy of the method developed.

CONCLUSION

A reverse phase HPLC isocratic method developed has been validated as per ICH guidelines in terms of specificity, accuracy, precision and linearity for the quantitative estimation of Balofloxacin in tablets. The precision is exemplified by relative standard deviation of 1.08%. A good linear relationship was observed for the drug between concentration ranges of 2.5 and 7.5 µg/ml. Accuracy studies revealed that mean recoveries were between 98 and 102%, an indicative of accurate method. Accordingly it can be concluded that the developed reverse phase isocratic HPLC method is accurate, precise and linear and therefore the method can be used for the routine analysis of Balofloxacin in tablets.

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CONFLICT OF INTERESTS

Declared None

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