Volume : 5 | Issue : 2 | February 2016 • ISSN No 2277 - 8179

Research Paper

# Comparative Study of RP-HPLC and UV Spectrophotometric Methods for Assay of Balofloxacin in Pharmaceutical Dosage Forms

Pharma

**KEYWORDS :** Balofloxacin; UV spectrophotometry; RP-HPLC; Validation

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## ABSTRACT

*Two simple, precise, accurate and economical RP-HPLC and UV spectrophotometric methods were developed and validated for the estimation of balofloxacin in the pharmaceutical dosage forms. The chromatographic separation was achieved isocratically on ACCLAIMTM 120 C18 column (5 µm, 4.6×250 mm) and methanol: 0.25 M phosphoric acid 80:20(v/v) as the mobile phase, at a flow rate of 1ml/min using isocratic elution. Detection was carried out at 293 nm. The retention time was found to be 2.7 min. The linearity range was found to be 5-50 µg/ml with r2 value of 0.9999. Accuracy was determined at 3 different levels by recovery studies and showed percent recovery ranging from 96.70-101.04%. UV spectrophotometric method was developed using 0.1 N HCl as the solvent and absorbance was recorded at λmax of 293 nm. Beer's law was obeyed in the concentration range of 2-14 µg/ml with r2 value of 0.9975. Accuracy was determined by recovery studies and showed percent recovery ranging from 99.92-101.20%. The developed methods can be used in QC laboratory for routine analysis to ensure the identity, purity, and performance of the drug product.*

## INTRODUCTION

Balofloxacin, 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylaminopiperidine-1-yl)-4-oxoquino line-3-carboxylic acid is a new generation fluoroquinolone antibiotic and has a broader spectrum of activity and reduced toxicity than other fluoroquinolones. It is prescribed for infective ophthalmitis, chronic bronchitis, community-acquired pneumonia, skin and urinary tract infections[1]. Its quantitative determination in pharmaceutical formulations is important to guarantee the desired therapeutic effects. Review of literature revealed that balofloxacin has been analysed by a RP-HPLC[2-6] and spectrophotometric[7-11] methods. The aim of the present study was to develop, validate and compare RP-HPLC and UVspectrophotometric methods for the analysis of balofloxacin in pharmaceutical formulations.

## Materials and Methods

***Reagents and chemicals:***

Gift sample of balofloxacin was provided by Cirex Pharmaeuticals (P) Ltd, Hyderabad, India. HCl LR, Methanol HPLC grade and milli Q water were used. Sample analysis was performed on Baloforce ™ 100 mg tablets purchased from local market.

**Instrumentation:**

Thermo Scientific HPLC, model Ultimate 3000 equipped with variable wavelength detector was used. Sample injection was manual, through the rheodyne injection valve with 20 µl loop. The output signal was monitored and integrated using Chromeleon software. UV method was performed on UV-visible double beam spectrophotometer, LABINDIA ANALYTICAL UV 3000+ and the output signal was monitored and integrated using UV WIN software.

**Chromatographic conditions:**

ACCLAIM TM C18 column (250×4.6 mm, 5 µ) was used for separation. The mobile phase containing methanol: 0.25 M phosphoric acid (80:20) was delivered at the flow rate of 1 ml/min with detection at wavelength 293 nm. The injection volume was 20 µl. The run time was 10 min.

**Spectrophotometric conditions:**

UV method was performed on UV Spectrophotometer, LABINDIA ANALYTICAL UV 3000+. Solvent used was 0.1 N HCl with detection at 293 nm.

**Standard stock solution:**

For HPLC method, Standard stock solution of balofloxacin was prepared by dissolving 25 mg in 25 ml mobile phase, methanol : 0.25 M phosphoric acid (80:20) v/v . From the stock solution, working standard was prepared by appropriate dilution to 10 ml with mobile phase.

For UV spectrophotometric method, Standard stock solution of balofloxacin was prepared by dissolving 10 mg in 10 ml 0.1 N HCl. Working standard was prepared by appropriate dilution to 10 ml with solvent.

***Selection of analytical wavelength*:**

For HPLC and UV methods, working standard solution prepared from the standard stock solution was scanned in the wavelength range from 200-400 nm to finalize the analytical wavelength for the present study.

**Application of the methods to the analysis of balofloxacin in the pharmaceuical dosage form:**

For HPLC method, Tablets, twenty in number (Baloforce ™) were finely powdered and an accurately weighed quantity of powder equivalent to 10 mg of balofloxacin was transferred to 10 ml volumetric flask and extracted with 10 ml mobile phase by shaking it for 15 min, filtered through whatmann No. 1 paper. From this solution, 0.2 ml was transferred to 10 ml volumetric flask and volume made up to obtain working sample solution of the drug.

For UV method, tablet powder equivalent to 10 mg of balofloxacin was transferred to 10 ml volumetric flask and extracted with 10 ml 0.1 N HCl by shaking it for 15 m, filtered through whatmann No. 1 paper. From this solution, 1 ml was transferred to 10 ml volumetric flask and volume made up to obtain working sample solution of the drug. From the working standard solution, 0.6 ml was diluted to 10 ml with the solvent and its absorbance was measured at the selected wavelength. The content of balofloxacin in sample solution of tablet was calculated using proposed method.

***Forced degradation studies*:**

For HPLC method, forced degradation studies for 8 hr duration were performed by subjecting the standard drug to acid hydrolysis (1N HCl) and base hydrolysis (1N NaOH) under reflux; elevat-

Volume : 5 | Issue : 2 | February 2016 • ISSN No 2277 - 8179

ed temperature 70$^0$C, oxidation (3% $H_2O_2$) and UV light.

**Method validation:**

The methods were validated as per ICH guidelines[12]. Specificity of the method was assessed by comparing the Rt and peak area of standard balofloxacin and sample, both injected at test concentration of 10 µg/ml. Linearity was determined for HPLC and UV method. The graph was obtained by plotting the area vs. concentration for HPLC and absorbance vs. concentration for UV method. Method accuracy for HPLC and UV was performed by spiking the known amounts of drug to the previously analyzed sample at three levels of 80 to 120% of the nominal analyte concentration. The percentage recovery in each case was calculated. Intra-day precision of six replicates on same day and on two different days for inter-day precision was done. Results are expressed as %RSD of the measurements. Robustness was determined by making small deliberate changes in the pH of the mobile phase ± 0.2 of the optimized pH, flow rate ±0.2 ml and mobile phase composition ±5% of the optimized ratio.

**RESULTS AND DISCUSSION**

The aim of this study was to develop and validate simple, accurate and sensitive RP-HPLC and UV spectrophotometric methods for the estimation of balofloxacin in pharmaceutical dosage forms. For HPLC method, chromatographic parameters such as mobile phase ratio, pH, and flow rate and detection wavelength were studied. Mobile phase containing methanol: 0.25 M phosphoric acid 80:20 (v/v) with a flow rate of 1 ml/min was selected as the optimized mobile phase since the drug chromatogram showed good peak shape, and symmetry with the Rt of 2.7 min. Since the λmax of the drug in the mobile phase was found to be 293 nm, it was selected as detection wavelength. For UV spectrophotometry λmax was determined as 293 nm.

Table 1: Validation parameters and Results of sample analysis

| Parameters | HPLC | UV Spectrophotometry |
|---|---|---|
| Theoretical plates | 4726 | -------- |
| Asymmetric factor | 1.13 | ------- |
| Retention Time (min) | 2.70 | --------- |
| Linearity (µg/ml) | 5-50 | 2-14 |
| Regression Equation | y = 1.9469x + 0.2162 | y = 0.0499x + 0.0288 |
| Slope (m) | 1.9469 | 0.0499 |
| Intercept (c) | 0.2162 | 0.0288 |
| Correlation coefficient | 0.9999 | 0.9975 |
| % RSD | Intraday-1.277631<br>Interday-0.980025 | Intraday-0.3875<br>Interday-1.2909 |
| Sample analysis (mg) (Label claim 100 mg) | 102.39 | 104.15 |

Table 2: Robustness data (HPLC method)

| Parameter | Optimized conditions | Variation | Observations | |
|---|---|---|---|---|
| | | | Rt (min) | % assay |
| Mobile phase Ratio | 80:20 | 75:25 | 2.920 | 102.10 |
| | | 80:20 | 2.710 | 98.86 |
| | | 85:15 | 2.617 | 100.15 |
| Mobile phase pH | 2.9 | 2.7 | 2.747 | 100.15 |
| | | 2.9 | 2.710 | 98.86 |
| | | 3.1 | 2.890 | 100.90 |
| Flow rate (in ml) | 1.0 | 0.8 | 2.230 | 106.67 |
| | | 1.0 | 2.710 | 99.70 |
| | | 1.2 | 3.333 | 94.44 |

Table 3: Stability profile ( from HPLC method)

| Stress degradation conditions | % Degradation of Balofloxacin |
|---|---|
| Thermal | 1.52 |
| Photo | 1.72 |
| Oxidation | 3.65 |
| Acid | 4.34 |
| Base | 1.10 |

For RP-HPLC method, system suitability parameters were determined using standard solutions and are summarized in Table 1. The values obtained suggest the suitability of the system for the analysis of the drug in the dosage form. For RP-HPLC and UV method, calibration curves were constructed by plotting the peak area (y-axis) to the concentration (x-axis) and absorbance at 293 nm (y-axis) to the concentration (x-axis) respectively. The response was found to be linear with a correlation coefficient of 0.999 and 0.9975 respectively, showing good correlation existing between area of the peak (or absorbance in UV analysis) and concentration. The robustness of the HPLC method was studied by changing the chromatographic conditions slightly and the results indicated that the proposed HPLC method was robust (Table 2). The results of forced degradation studies are presented in Table 3. The developed methods were successfully applied for the determination of balofloxacin in the pharmaceutical dosage form and the results of the assay were comparable with the corresponding labeled amounts. High recovery values (HPLC and UV) and no additional peaks in the chromatogram indicate that the developed methods are accurate and specific and can be used for the routine analysis of balofloxacin in the pharmaceutical dosage forms.

**CONCLUSION**

The RP-HPLC method gives good peak shape, asymmetry with a short analysis time. Both the methods are validated and are simple, sensitive, accurate and precise. The results obtained from UV spectrophotometric method using 0.1 N hydrochloric acid as solvent was comparable to the results of HPLC analysis of balofloxacin. Also the degradation ability of balofloxacin was extremely negligible and under stress conditions was demonstrated to be under 4% indicating the usefulness of UV spectrophotometric method. This indicates that the UV spectrophotometric using 0.1 N hydochloric acid method for analysis of balofloxacin is comparable to HPLC method and could be best choice in routine analysis of balofloxacin tablets in routine analysis.

**ACKNOWLEDGEMENTS**

Authors wish to thank Cirex Pharmaeuticals (P) Ltd, Hyderabad, India for providing the gift sample of balofloxacin. The authors are also thankful to Dr. G.K. Rao, Principal, Goa College of Pharmacy for supporting research activities at the department.

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