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SPECTROPHOTOMETRIC QUANTIFICATION OF BALOFLOXACIN USING MULTIVARIATE TECHNIQUE IN PHARMACEUTICAL FORMULATION

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ABSTRACT

The study was focused to develop and validate a simple, sensitive and accurate UV spectrophotometric method with multivariate calibration for the quantification of balofloxacin in pharmaceutical dosage form. This technique was based on the use of the linear regression equations by using relationship between concentration and absorbance at five different wavelength sets. The developed method was validated for accuracy, precision and sensitivity according to ICH guidelines. The plot was found to be linear in the concentration range of 10-50 µg/mL with significantly high value of correlation coefficient. The linear regression analysis data for the calibration plots showed good linear relationship with $R^2 = 0.9999$. The regression equation was found to be Y = 0.0114 X + 0.0885. The LOD and LOQ were found to be 1.38 and 4.11 µg/mL, respectively and the method was found to be sensitive. This statistical approach gives optimum results for eliminating fluctuations coming from instrumental or experimental conditions and it also has considerable sensitivity, rapid, accuracy and low cost for the quantitative analysis, quality control and routine analysis of balofloxacin in pharmaceutical formulation.

Key words: Balofloxacin, Quinoline antibiotics, UV Spectrophotometer, Multivariate calibration.

INTRODUCTION

Balofloxacin [1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylaminopiperidin-1-yl)-4-oxoquinoline-3-carboxylic acid][1,2] is a third generation broad spectrum flouroquinolone antibiotic acts by interrupting the enzyme DNA gyrase; thereby, inhibits DNA synthesis in bacteria. It is indicated in cause of infective ophthalmitis and sinusitis, chronic bronchitis, acute exacerbation, skin infections and intra abdominal infections.

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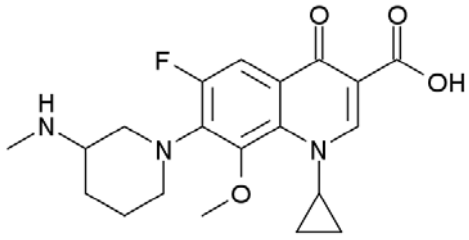

Fig. 1: Chemical structure of balofloxacin

Multivariate calibration represents the transition of common single species analysis from one dependent variable (measured value, or measurand) to *m* dependent variables, e.g., wavelengths or sensors, which can be simultaneously included in the calibration model. It is possible to determine $n \geq 1$species in the analytical system[3].

Under optimized conditions, the applied numerical method provides considerable resolving power, sensitivity, rapidity, and low cost for the quantitative analysis, quality control and routine analysis of subject compounds. The mathematical algorithm of this approach is explained as follows:

If the absorbance of an analyte (X) is measured at five wavelengths set (λ = 280, 283, 286, 289, and 292 nm), following equations can be written for each data set,

$$A\lambda 260 = a \times C_x + k1 \quad ...(1)$$

$$A\lambda 265 = b \times C_x + k2 \quad ...(2)$$

$$A\lambda 270 = c \times C_x + k3 \quad ...(3)$$

$$A\lambda 275 = d \times C_x + k4 \quad ...(4)$$

$$A\lambda 280 = e \times C_x + k5 \quad ...(5)$$

where, Aλ represent the peak area of the analyte; a, b, c, d and e are the slopes of linear regression functions of the analyte; k1, k2, k3, k4 and k5 are the intercepts of linear regression functions at five selected wavelengths and C_x represents the concentration of analyte. The five-equation system (1-5) can also be rewritten as:

$$AT = a \times C_x + b \times C_x + c \times C_x + d \times C_x + e \times C_x + KT \quad \ldots(6)$$

which can b e simplified as -

$$AT = C_x (a + b + c + d + e) + KT \quad \ldots(7)$$

where, AT and KT represents the sum of peak area obtained and sum of intercepts of regression equations at five-wavelength set respectively. The concentration of the X analyte in a solution of unknown concentration can be calculated by using the following equation:

$$C_x = \frac{AT - KT}{(a + b + c + d + e)} \quad \ldots(8)$$

In this case, the multivariate chromatographic calibration mood contains the use of linear regression function based on the relationship between peak area ratio and concentration mood is replaced for the prediction of unknown concentration of analyte[4].

Literature review reveals that balofloxacin was estimated by HPLC-FID[5], HPLC-ESI-MS[6], fluorescent spectroscopy[7] and UV[8] and derivative spectroscopy[9]. It was found that no UV spectrophotometric multivariate technique has been reported for the estimation of balofloxacin in formulations. The objective of the work was to develop simple, sensitive, fast, reliable, multivariate calibration technique, i.e., use of the linear regression equations by using relationship between concentration and absorbance at five different wavelengths for the quantitative determination of balofloxacin in pharmaceutical formulation.

EXPERIMENTAL

Materials and methods

Chemicals and solvents

- Distilled water.

Solubility

- Very freely soluble in water.
- Soluble in 0.1 N HCl.
- Soluble in 0.1 N NaOH.
- Soluble in alcohol.

Instrument

- Perkin-Elmer UV-Visible spectrophotometer
- Sonicator
- Digital balance.

Method development

Selection of solvent

Balofloxacin was freely soluble in water. So water was chosen as a solvent to solubilise the raw material and sample to carry out the research work.

Preparation of standard stock solution

The standard stock solution of balofloxacin was prepared by dissolving 100 mg in 100 mL of water to get a concentration of 1 mg/1 mL.

Preparation of sample solution

Twenty balofloxacin tablets were powdered in a motor and an equivalent amount of 100 mg of drug was dissolved in 100 mL distilled water to make a solution (1 mg/mL), which was further filtered and diluted in the working concentration range of 10-50 µg/mL. Absorbance versus concentration was plotted, which gave a linear plot.

Determination of λ_{max}

The stock solution of balofloxacin was diluted with distilled water to get a concentration of 10 µg/mL. The solution was scanned in the UV region from 200-400 nm. Five different wavelengths were selected fixing the λ_{max} (286 nm) as the center wavelength.

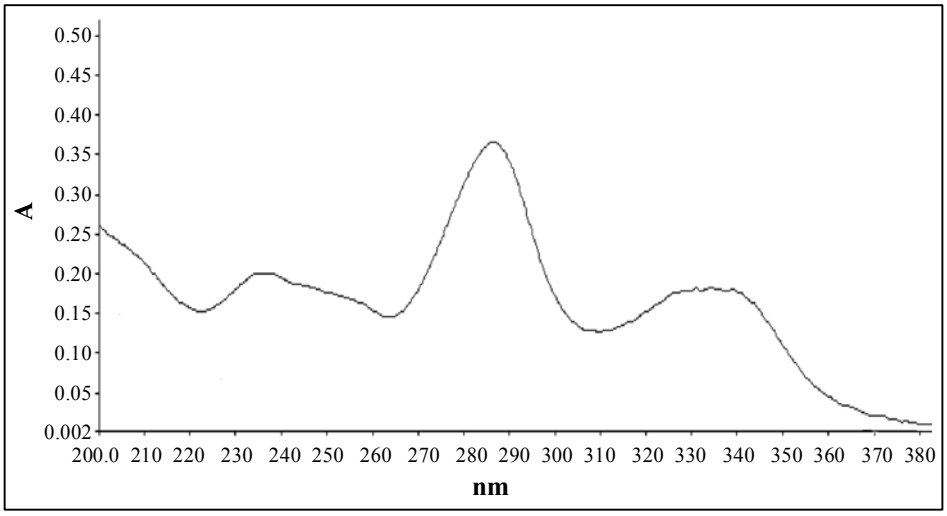

Fig. 2: UV Spectrum of balofloxacin at 286 nm.

Table 1: Multivariate UV calibration obtained at five wavelengths

Concentration (µg/mL)	Absorbance				
	280	283	286	289	292
10	0.1754	0.1877	0.2051	0.1739	0.1697
20	0.285	0.2961	0.3164	0.2909	0.2848
30	0.3861	0.4049	0.426	0.4014	0.3893
40	0.496	0.5233	0.5442	0.5237	0.5067
50	0.6066	0.6337	0.6566	0.6328	0.6143

Method validation[10,11]

The method was validated according to International Conference on Harmonization (ICH) Q2B complete guidelines for linearity, sensitivity, precision and accuracy.

Linearity

Stock solution of balofloxacin was diluted with distilled water to get a concentration ranging from 10-50 µg/mL. The absorbance of the above solutions was measured over a range surrounding 286 nm i.e., 280, 283, 286, 289 and 292 nm in order to improve the correlation and minimize instrumental fluctuations. The absorbance of the different concentration solutions were recorded at five different wavelengths and the calibration curves were constructed (Fig. 2).

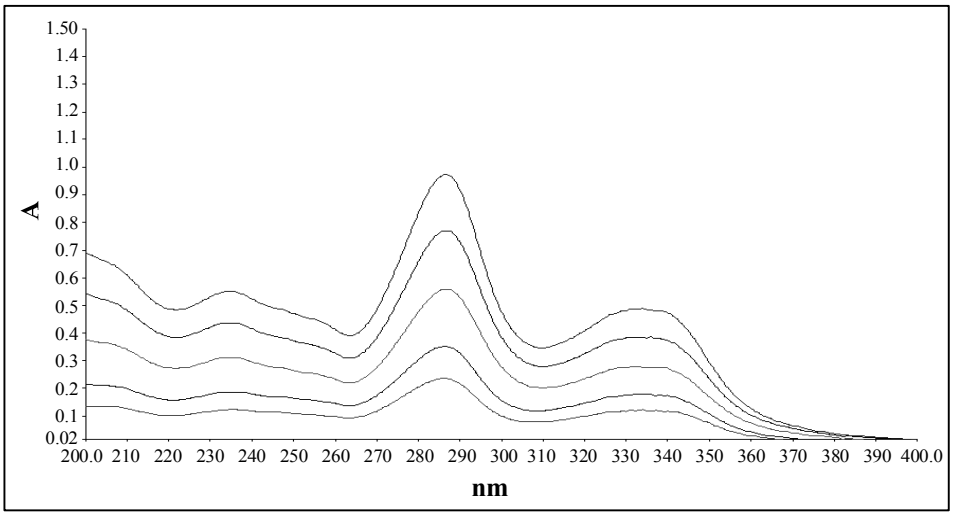

Fig. 2: UV Spectrum showing linearity of balofloxacin at 286 nm.

Sensitivity for the method was determined by calculating LOD and LOQ by using the formulae -

$$LOD = 3.3\ \sigma/S \text{ and} \quad \ldots(9)$$

$$LOQ = 10\ \sigma\ /S \quad \ldots(10)$$

Where, σ is the standard deviation of the lowest standard concentration and

S is the slope of the standard curve.

Precision

From the prepared standard stock solution, 10 mL of the solution was diluted to 100 mL using water. From the above solution, 3 mL of solution was pipetted out into 10 mL volumetric flasks and the volume was made with water to get the final concentrations of 30 µg/mL. These aliquots were scanned at all five wavelengths using UV spectrophotometer six times a day i.e., inter-day precision. The aliquots are scanned on six days at same time using UV spectrophotometer for intra-day precision.

Accuracy

Accuracy of the developed method was determined by standard addition method. From the prepared stock solutions of standard and sample, 10 mL of the solutions were pipetted out and diluted to 100 mL to get a concentration of 100 µg/mL. From standard solution, 10 mL was pipetted into 3 different volumetric flasks and the diluted sample solution of 0 mL, 20 mL and 40 mL was added to the above 3 volumetric flasks containing standard solutions and the volume were made up to 100 mL with water. These aliquots were scanned using UV spectrophotometer and the % recovery was calculated at all the five wavelengths.

RESULTS AND DISCUSSION

The λ_{max} of balofloxacin was found to be 286 nm using distilled water as solvent. All the calibration curves prepared were linear over the concentration range 10-50 µg/mL. The linear regression analysis data for the calibration plots showed good linear relationship with $R^2 = 0.9999$. The regression equation was found to be $Y = 0.0114\ X + 0.0885$. The %RSD for precision was found to be 0.30-1.30 and the LOD and LOQ were found to be in the range

of 0.95-1.35 µg/mL and 2.89-4.11 µg/mL, respectively at all the five wavelengths, which were within the acceptance limits according to ICH guidelines.

Linearity

The linearity for different concentrations 10-50 µg/mL were recorded at 280, 283, 286, 289 and 292 nm, which were shown in Fig. 2 for n (n = 6) number of sets and their calibration graphs are shown in the Figs. 3 to 6, respectively.

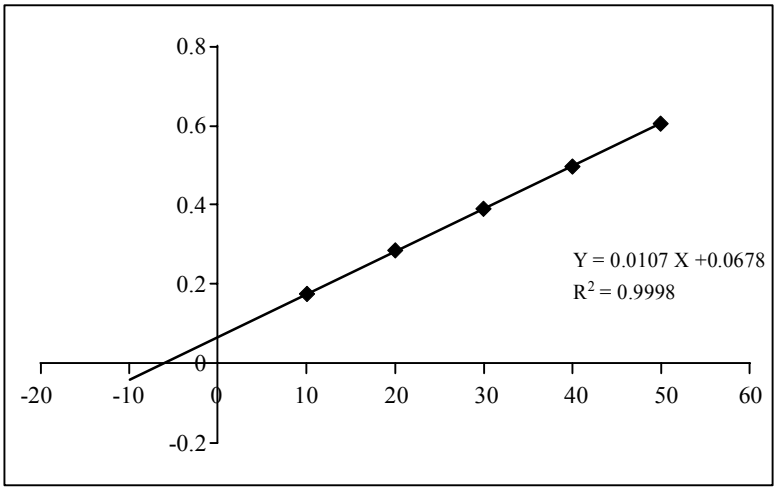

Fig. 3: Calibration graph of balofloxacin at 280 nm.

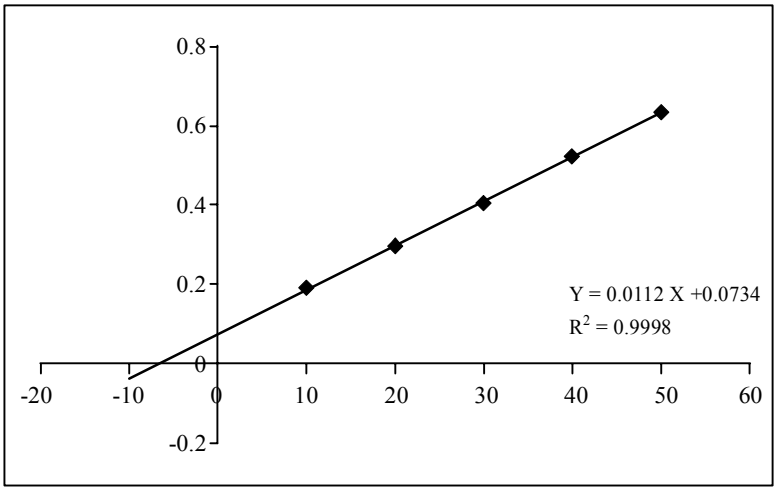

Fig. 4: Calibration graph of balofloxacin at 283 nm.

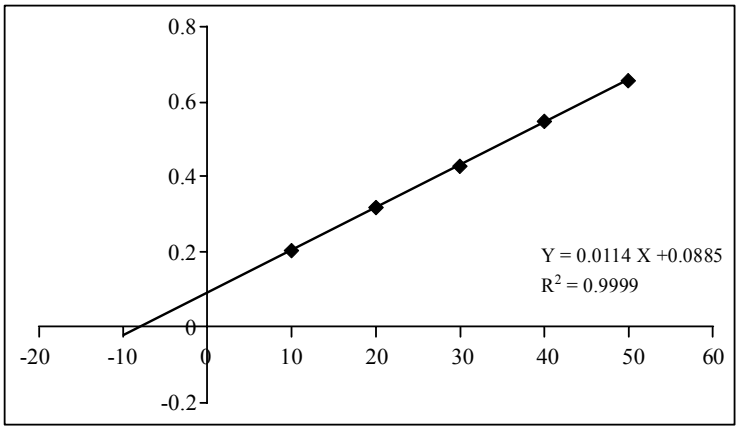

Fig. 5: Calibration graph of balofloxacin at 286 nm.

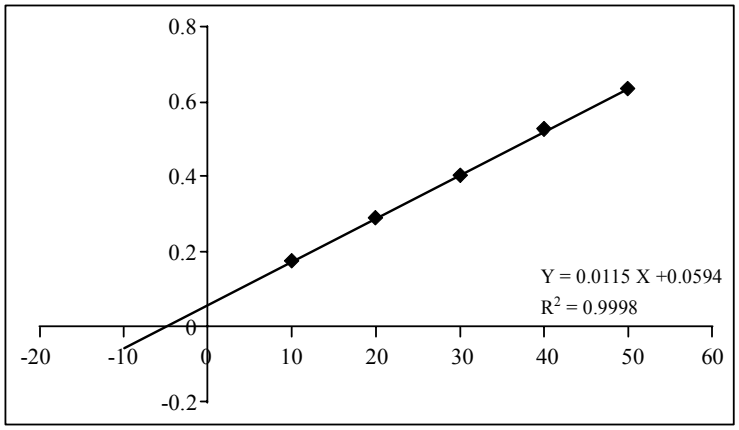

Fig. 6: Calibration graph of balofloxacin at 289 nm

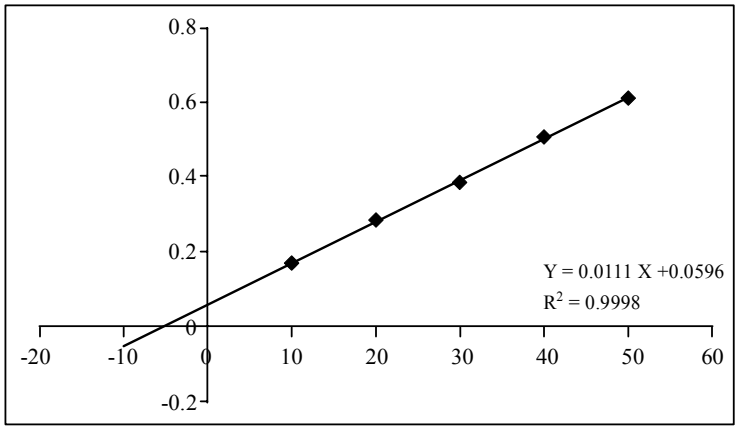

Fig. 7: Calibration graph of balofloxacin at 292 nm

The low value of SD at all wavelengths indicates that the method was precise and the LOD and LOQ were calculated and tabulated in Table 2.

Table 2: Linearity data showing LOD and LOQ at all the five wavelengths

Wavelength (nm)	Regression equation	R^2	SD	LOD (µg/mL)	LOQ (µg/mL)	% RSD
280	Y = 0.0107x + 0.0678	0.9998	0.0031	0.9560	2.8971	1.27
283	Y = 0.0112x + 0.0734	0.9998	0.0045	1.3878	4.2056	1.06
286	Y = 0.0113 x + 0.0904	0.9999	0.0044	1.3570	4.1121	1.04
289	Y = 0.0115 x + 0.0594	0.9998	0.0053	1.6345	4.9532	1.14
292	Y =0.0111 x + 0.0596	0.9998	0.0044	1.3570	4.1121	1.26

Precision

The low value of SD at all wavelengths indicates that the method was precise and the % RSD for intra-day and inter-day precision were found to be 0.30-1.30 and well within acceptance criteria less than 2% at all the five wavelengths. The low value of %RSD indicates the proposed method was accurate.

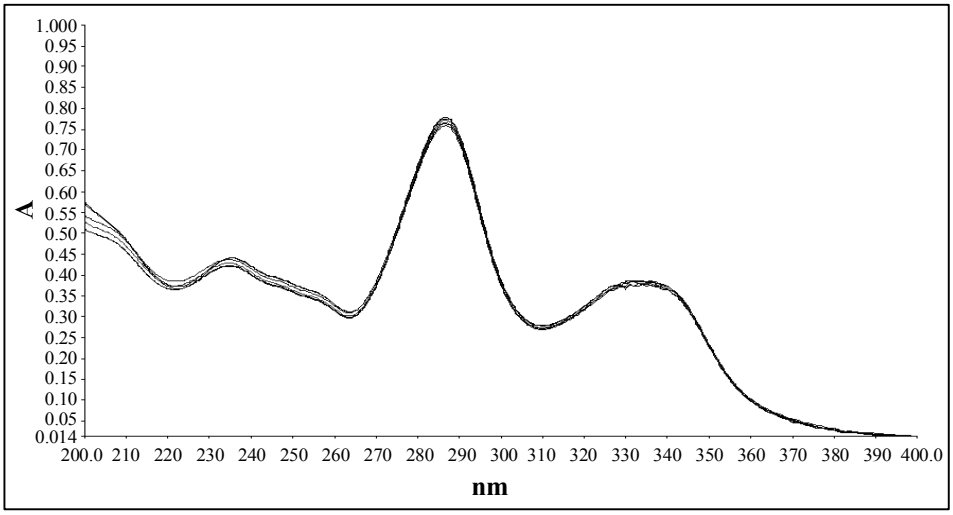

Fig. 8: UV Spectrum showing intra-day precision of balofloxacin

Table 3: Intra-day precision

Days	Wavelength (nm)				
	280	**283**	**286**	**289**	**292**
1	0.5543	0.6516	0.7173	0.6662	0.5376
2	0.5497	0.657	0.7226	0.6549	0.5284
3	0.5578	0.649	0.7103	0.6618	0.5233
4	0.5506	0.6599	0.7214	0.6491	0.539
5	0.5627	0.6581	0.7255	0.6699	0.5212
6	0.5607	0.6604	0.7285	0.6634	0.5327
% RSD	0.9583	0.7095	0.8932	1.1551	1.3858

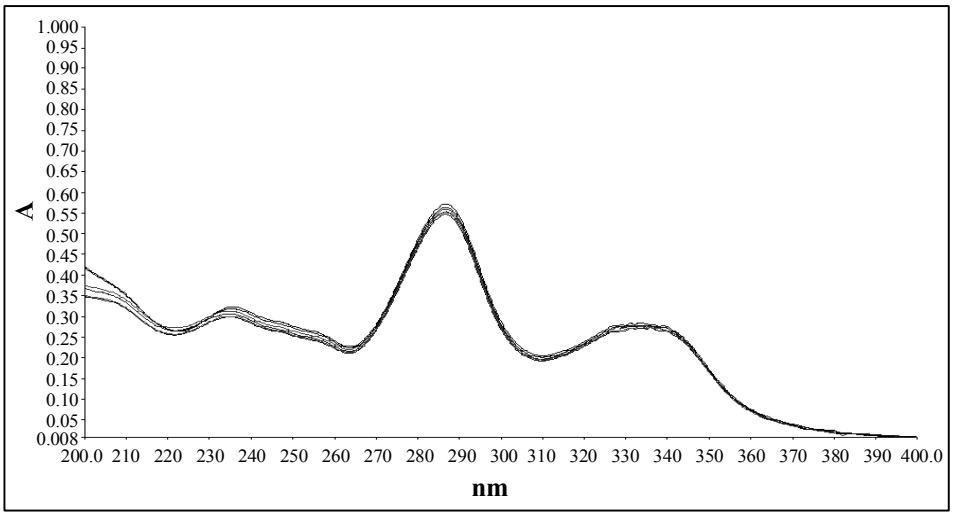

Fig. 9: UV Spectrum showing inter-day precision of balofloxacin

Table 4: Inter-day precision

Wavelength (nm)	Absorbance						% RSD
	1	**2**	**3**	**4**	**5**	**6**	
280	0.3409	0.3362	0.3389	0.3358	0.3464	0.3431	1.2075
283	0.3915	0.3956	0.3836	0.3875	0.3843	0.3823	1.3365

Cont…

Wavelength (nm)	Absorbance						% RSD
	1	2	3	4	5	6	
286	0.4139	0.4095	0.4278	0.4152	0.4219	0.4164	1.5483
289	0.3851	0.3883	0.3757	0.3819	0.3846	0.376	1.3439
292	0.3689	0.3615	0.3594	0.3701	0.365	0.3673	1.1567

Recovery

The percentage recovery of drug from synthetic mixture was found to be in the range of 97.53 – 102.87 % w/w, which was well within the acceptance limit 97-103% w/w as per ICH guidelines.

Table 5: Recovery studies

Wavelength (nm)	Amount present (µg/mL)	Amount added (µg/mL)	Absorbance	Amount recovered (µg/mL)	% Recovery
280	10	0	0.1757	9.9829	99.82
		20	0.3849	30.0935	100.93
		40	0.6073	49.9423	99.42
283	10	0	0.1861	9.9247	99.24
		20	0.4067	30.1333	101.33
		40	0.6319	49.8579	98.57
286	10	0	0.2077	10.1267	101.26
		20	0.4281	30.1478	101.47
		40	0.6541	49.8096	98.09
289	10	0	0.1787	10.2878	102.87
		20	0.4039	30.1868	101.86
		40	0.6307	49.834	98.34
292	10	0	0.1724	10.1591	101.59
		20	0.3861	29.7534	97.53
		40	0.6126	49.8616	98.61

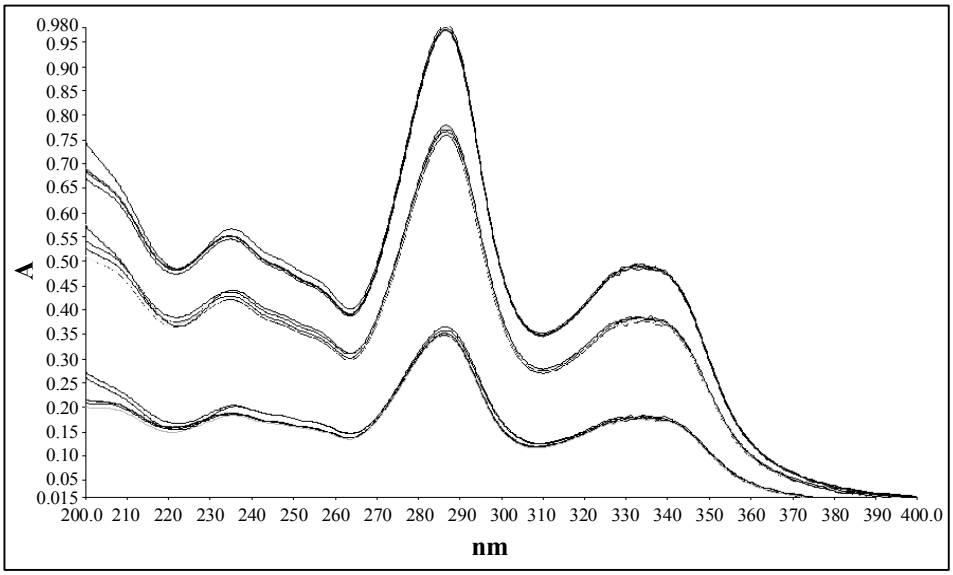

Fig. 10: UV Spectrum showing accuracy of balofloxacin

CONCLUSION

The newly developed spectrophotometric multivariate calibration technique was validated by evaluating various validation parameters and was found to be within the prescribed limits. The method developed in the study was found to be sensitive, accurate, precise and reproducible for determination of balofloxacin in tablet formulation. Therefore, the developed spectrophotometric multivariate calibration method can be recommended for routine quality control analysis of balofloxacin in pharmaceutical formulations.

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