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DEVELOPMENT AND VALIDATION OF A SIMPLE UV SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF LEVOFLOXACIN BOTH IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT: The present study was undertaken to develop and validate a simple, accurate, precise, reproducible and cost effective UV-Visible spectrophotometric method for the estimation of levofloxacin in bulk and pharmaceutical formulation. The solvent used throughout the experiment was 0.1N NaOH. Absorption maximum (λ_{max}) of the drug was found to be 288 nm. The quantitative determination of the drug was carried out at 288 nm and Beer's law was obeyed in the range of 2-12µg/mL. The method was shown linear in the mentioned concentrations having line equation y = 0.0704x + 0.0034 with correlation coefficient $R^2$of 0.999. The recovery values for levofloxacin ranged from 99.92% - 100.33%. The relative standard deviation of six replicates of assay was less than 2%.The percent relative standard deviation (RSD %) of interday precision range was 0.469 – 0.925 % and intraday precision range was 0.685 – 0.713%. The limit of detection and limit of quantification was 0.087µg/mL and 0.164µg/mL. The percent relative standard deviation of robustness and ruggedness of the method was 0.136 - 0.213%. Hence, proposed method was precise, accurate and cost effective. This method could be applicable for quantitative determination of the bulk drug as well as dosage formulation.

INTRODUCTION: Levofloxacin is a broad spectrum second generation fluoroquinolone antibacterial agent, structurally related to nalidixic acid [1]. Its spectrum of activity includes most strains of bacterial pathogens responsible for respiratory, urinary tract, gastrointestinal, and abdominal infections, including gram negative, gram positive and some atypical bacterial pathogens [2-9]. Levofloxacin stops multiplication of bacteria by preventing the reproduction and repair of their genetic material [10].

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Literature survey revealed that few analytical methods are available for the individual estimation of levofloxacin by HPTLC [11-12], by HPLC [13-14] and by conductometry [15]. Recently some UV spectrophotometric methods were reported for the estimation of levofloxacin using various solvent such as 0.1M HCl [16], 100% methanol [17], acetonitrile [18] and the mixer of water, methanol and acetonitrile [19]. But single estimation of this drug with 0.1M sodium hydroxide as solvent has not been reported in bulk and in pharmaceutical formulation.

Thus, the aim of the present work was to develop and validate a simple, reproducible and economic analytical method to estimate levofloxacin in routine analysis.

MATERIALS AND METHOD:

Materials: Pure Standard of levofloxacin in the form of levofloxacin hemihydrates powder was received as a kind gift from Renata Pharmaceuticals Ltd., Bangladesh which was used as reference standard. The commercial levofloxacin dosage forms used were tablet levoxin-500mg and infusion (IV) levoxin (100 ml, 5mgmL^{-1}) were purchased from the local market.

Apparatus: A Shimadzu UV-Visible spectrophotometer UV-1800 was used.

Method development:

1. **Determination of λ_{max}:** The standard stock solution of 100 µg/mL of levofloxacin was prepared by weighing 100 mg of the drug, taken in 100 mL volumetric flask and diluted with 0.1N NaOH. By appropriate dilution of standard stock solutions with diluent, different solutions containing different concentration (2, 4, 6,8,10 & 12µg/mL) of levofloxacin were scanned in the range of 200-800 nm to determine the wavelength of maximum absorbance. Levofloxacin has shown maximum absorption at 288 nm.

Method Validation: The proposed method was validated for different parameters like linearity, precision, accuracy, specificity, robustness, LOD, LOQ and assay.

2. **Linearity:** The linearity was determined by plotting concentration against corresponding absorbance. Standard stock solutions, 100µg/mL were further diluted to obtain 2µg/mL-12µg/mL solutions. The calibration curves were constructed by plotting absorbance versus concentration and the regression equations were calculated.

3. **Precision:** The system precision is a measure of the method variability. It was determined by performing three replicate analyses of the same working solution. Precision of the method was demonstrated by intraday and interday variation studies. The intraday precision of the developed UV method was determined by preparing the samples of the same batch in nine determinations with three concentrations (2, 4, 6 µg/mL) and three replicate (n=3) each on same day i.e. zero hour, fourth hour and eighth hour. The percentage RSD of the results was used to evaluate the method precision. The interday precision was determined by assaying the samples in triplicate (n=3) per day for consecutive 3 days.

4. **Accuracy:** Accuracy of the method was calculated by recovery studies at three levels (80%, 100% and 120%) by standard addition method. An accurately weighed tablet powder equivalent to 10mg of levofloxacin was diluted with 0.1N NaOH to make100 µg/mL and was sonicated for 20 minutes. The solution was then filtered through a Whatmann filter paper (No. 41). From this solution, 1mL was transferred to three 10 mL volumetric flasks and 0.8 mL (Flask 1), 1 mL (Flask 2), and 1.2 mL (Flask 3) of stock solution of API was added and then it was made up to the mark with diluent to make them 80%, 100% and 120% spiking.

5. **Specificity in the presence of excipients:** The specificity test was carried out using only excipients. Spectra for placebo granules, blank and sample were measured and compared. The sample solution was kept in the oven (60^0C) and under the UV lamp (254 nm) for 72h in order to verify that none of the degradation products interfered with the quantification of the drug.

6. **Robustness:** The robustness of an analytical procedure is the measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It was determined by carrying out the analysis by two analysts at two different temperatures i.e. at 20^0C and 30^0C. The absorbance was measured and assay was calculated for six times.

7. **Limit of detection (LOD) and limit of quantitation (LOQ):** LOD and LOQ were calculated from the data obtained from the linearity studies. The slope of the linearity plot was determined. For each of the ten replicate determinations of same concentration (6µg/mL), standard deviation (SD) of the responses was calculated. From these values, the limit of detection and limit of quantitation were determined on the basis of standard deviation and slope of the regression equation.

Assay of levofloxacin formulations available in Bangladesh: Twenty tablets were individually weighed and triturated to obtain homogeneous mixture. An amount of powder equivalent to 10mg of levofloxacin was taken in 100mL volumetric flask and was diluted with 0.1N NaOH to make 100µg/mL and was sonicated for 20 min to effect complete dissolution of levofloxacin, the solution was then filtered. The aliquot of the filtrate was further diluted to get final concentration of 10µg/mL of levofloxacin. The % assay of the drug was calculated.

To analyze the concentration of levofloxacin injection, 2mL of levofloxacin infusion (which contain 5 mg mL^{-1}) was transferred in 100ml volumetric flask and was diluted with 0.1N NaOH. This solution was further diluted to get final concentration of 10µg/mL of levofloxacin. The % assay of the drug was calculated. All determinations were conducted by thrice time.

Statistical analysis: The results were expressed as mean±SD. Some results were expressed as %RSD.

RESULTS AND DISCUSSION: The method discussed in the present work provides a convenient and accurate way for analysis of levofloxacin. The different concentrations of 2, 4, 6, 8, 10 and 12 µg/mL were scanned and the wavelength of maximum absorption was found at 288 nm (**Figure 1**).

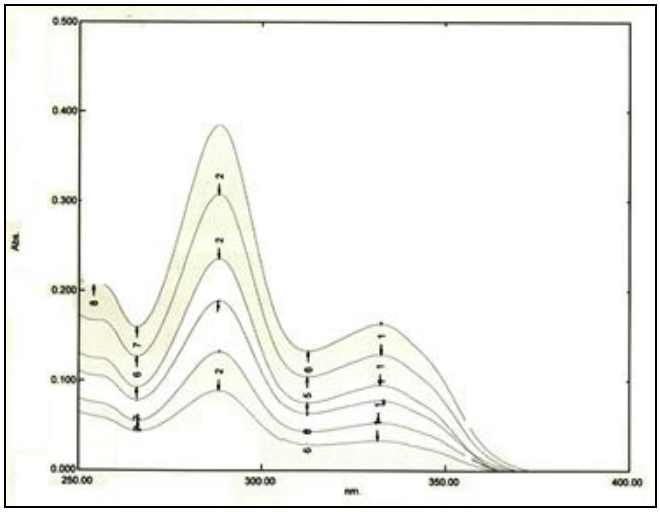

FIGURE 1: UV SPECTRUM OF LEVOFLOXACIN (λ_{max})

The drug obeyed the Beer's law with the concentration range 2– 12 µg/mL with R^2 value 0.999 and represented excellent linear relationship of the newly developed method (**Figure 2**). The LOD and LOQ of the developed method were determined by injecting progressively low concentrations of the standard solutions for 6 times and the values of LOD and LOQ were found to be as 0.087µg/mL and 0.164µg/mL respectively.

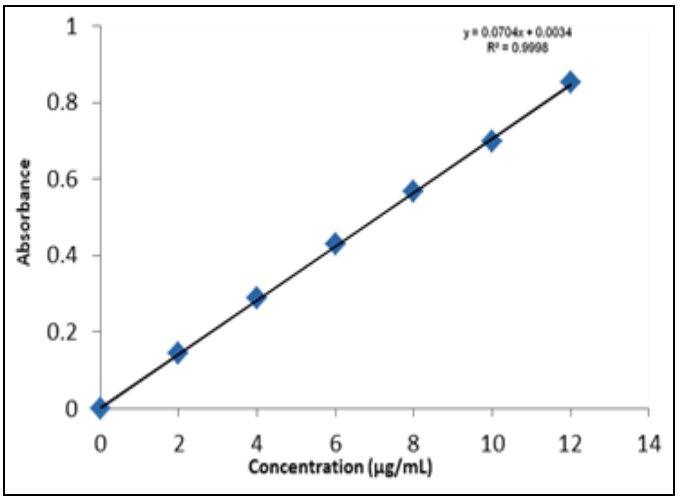

FIGURE 2: CALIBRATION CURVE FOR LEVOFLOXACIN

The precision of the proposed method was checked by intra-day and inter-day repeatability of responses which confirmed adequate sample stability and method reliability over 24 h periods where RSD% amongst responses was found as < 2% (**Table 1**).

TABLE 1: INTRA-DAY AND INTER-DAY PRECISION OF ASSAY UV-VISIBLE SPECTROPHOTOMETER

Concentration (µg/mL)	Absorbance			SD	% RSD
	0 hour	4 hour	8 hour		
Inter-day precision					
2	0.145	0.143	0.144	0.001	0.694
4	0.289	0.285	0.284	0.003	0.925
6	0.428	0.426	0.424	0.002	0.469
Intra-day precision					
2	0.147	0.146	0.145	0.001	0.685
4	0.292	0.291	0.288	0.002	0.711
6	0.431	0.429	0.425	0.003	0.713

The accuracy was evaluated at three different concentrations which were conducted in successive analysis (n = 3) using the proposed method and the value was expressed as percentage of recovery between the mean concentrations of recovered and injected concentration of the drug. The average recoveries were found to be as 99.96%, 100.33% and 99.92% for the concentration levels of 80%, 100% and 120% respectively (**Table 2**).

TABLE 2: DETERMINATION OF ACCURACY OF LEVOFLOXACIN BY UV-VISIBLE SPECTROPHOTOMETER (n=3)

% Recovery	Concentration (µg/mL)			% Recovery	Avg. Recovery	% RSD
	Formulation	Drug added	Drug found			
80	10	8	7.99	99.88		
80	10	8	8.02	100.25	99.96%	0.260
80	10	8	7.98	99.75		
100	10	10	10.01	100.1		
100	10	10	10.05	100.5	100.33%	0.259
100	10	10	10.04	100.4		
120	10	12	12.01	100.08		
120	10	12	11.99	99.92	99.92%	0.169
120	10	12	11.97	99.75		

The assays were validated by means of the analysis of variance. Levofloxacin content in two pharmaceutical dosage forms (tablet and injection) were determined by this proposed method which were in good agreement with the label claims with RSD value of 0.02% for tablet and 0.01% for injectable dosage form (**Table 3**). The %RSD was found in the range of 0.136 – 0.213% for robustness and ruggedness. The specificity of the analytical method was proved by comparing the spectra of placebo and degradation product of sample solution with that of accuracy sample.

TABLE 3: ASSAY OF LEVOFLOXACIN IN MARKETED PRODUCTS (n=3)

Pharmaceutical Dosage Form	Declared concentration	Found concentration	Content (%)	% RSD
Tablet (500 mg)	10	9.99 ± 0.02	99.96% ±0.02	0.02
Injection 100mL (5mg mL^{-1})	10	9.98 ± 0.01	99.85% ± 0.01	0.01

All experimental results were within the range of the acceptability which indicated that the developed method was sensitive enough and accurate for quantitative analysis of levofloxacin.

Therefore, the method was applied for quantitative analysis of levofloxacin in bulk and pharmaceutical dosage form.

CONCLUSION: This UV-spectrophotometric technique was quite simple, accurate, precise, reproducible and sensitive. The UV method has been developed for quantification of levofloxacin in pharmaceutical dosage forms. The validation procedure confirms that this is a workable method for their quantification in the raw material and also in the formulations.

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