



## SHORT COMMUNICATION

# Development and validation of a simple UV spectrophotometric method for the determination of levofloxacin both in bulk and marketed dosage formulations

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**Abstract** A rapid, specific and economic UV spectrophotometric method has been developed using a solvent composed of water:methanol:acetonitrile (9:0.5:0.5) to determine the levofloxacin content in bulk and pharmaceutical dosage formulations. At a pre-determined  $\lambda_{\max}$  of 292 nm, it was proved linear in the range of 1.0–12.0  $\mu\text{g/mL}$ , and exhibited good correlation coefficient ( $R^2=0.9998$ ) and excellent mean recovery (99.00–100.07%). This method was successfully applied to the determination of levofloxacin content in five marketed brands from Bangladesh and the results were in good agreement with the label claims. The method was validated statistically and by recovery studies for linearity, precision, repeatability, and reproducibility. The obtained results proved that the method can be employed for the routine analysis of levofloxacin in bulks as well as in the commercial formulations.

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## 1. Introduction

Levofloxacin or L-ofloxacin, the bacteriologically active L-isomer of the racemic fluoroquinolone ofloxacin, is a broad-spectrum

antimicrobial agent. Levofloxacin acts by inhibiting bacterial DNA gyrase which is required for DNA replication and thus causes bacterial lysis [1]. The addition of 6-fluoro and 7-piperazinyl groups to the molecule greatly increases their antibacterial activity. They are commonly referred to as the second generation fluoroquinolone antibacterial agents and are greatly effective against both gram-negative and gram-positive bacteria that are resistant to other antibacterials [2–4].

Several HPLC assay methods have been reported for the determination of ofloxacin or its stereoisomers [5–9]. Literature survey revealed that various analytical methods such as high performance thin layer chromatography (HPTLC) [10] and conductometry [11] have been reported for the estimation

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of levofloxacin. Recently some UV spectrophotometric methods were also reported for estimating levofloxacin using various solvents like 0.1 M hydrochloric acid [12], 100% methanol [13] or acetonitrile [14].

In this study, efforts were made to develop a simple, easy and economic UV spectrophotometric method using a diluent composed of water:methanol:acetonitrile (9:0.5:0.5) for the determination of levofloxacin in the raw materials as well as in the marketed dosage formulations. The developed method was optimized and validated as per the guidelines of International Conference on Harmonization (ICH) [15] and demonstrated excellent specificity, linearity, precision and accuracy for levofloxacin.

## 2. Materials and methods

### 2.1. Apparatus

A Shimadzu UV–visible spectrophotometer (UV mini-1700, Shimadzu Corporation, Kyoto, Japan) was used for all absorbance measurements with matched quartz cells.

### 2.2. Materials

All chemicals and reagents were of analytical or HPLC grade. Levofloxacin in the form of levofloxacin hemihydrate powder was provided by Incepta Pharmaceuticals Ltd., Bangladesh, which was used as the reference standard. Pharmaceutical grade excipients were obtained from Pharmaceutical Technology Lab. of State University of Bangladesh.

### 2.3. Determination of wavelength of maximum absorption

A standard stock solution ( $L_S$ ) of levofloxacin (20  $\mu\text{g}/\text{mL}$ ) was prepared using diluents and 3 mL of  $L_S$  was then diluted to 10 mL with the same diluent to obtain 6  $\mu\text{g}/\text{mL}$  levofloxacin reference solution ( $L_R$ ). An UV spectroscopic scanning (190–400 nm) was carried out with the  $L_R$  to determine the  $\lambda_{\text{max}}$  for the detection of levofloxacin using diluent as blank.

### 2.4. Linearity and range

For linearity study, seven solutions at different concentrations (1, 2, 4, 6, 8, 10 and 12  $\mu\text{g}/\text{mL}$ ) were prepared using seven different aliquots of  $L_S$ , and the obtained data were used for the linearity calibration plot. Limit of detection (LOD) and limit of quantification (LOQ) for the assay were also calculated [15].

### 2.5. Intra-day precision (repeatability) and inter-day precision study (intermediate precision)

Levofloxacin tablets were finely powdered and the sample stock solution ( $L_P$ ) of 20  $\mu\text{g}/\text{mL}$  was prepared following the same dilution pattern of  $L_S$ . Three different aliquots of  $L_P$  were then diluted to 10 mL to obtain the concentrations of 4, 6 and 8  $\mu\text{g}/\text{mL}$ . This procedure was repeated in the following days.

### 2.6. Stability study

Samples prepared for repeatability study were preserved for 24 h at room temperature and analyzed on the following day to test for short-term stability.

### 2.7. Accuracy/recovery study

This study was carried out using pre-formulated granules containing pure levofloxacin hemihydrate and common excipients. Calculation was done from the label claim and the average weight of the final product. Previously used dilution pattern was followed for the granules to obtain five concentrations—80%, 90%, 100%, 110% and 120% of reference solution.

### 2.8. Specificity in the presence of excipients

The test for the specificity was carried out using only excipients. Spectra for placebo granules, blank, and sample were compared. Secondly the specificity was determined by subjecting the sample solution to accelerated degradation by heat (60 °C) for 72 h in order to verify that none of the degradation products interfered with the quantification of the drug.

### 2.9. Assay of content of levofloxacin in selected marketed brands

Five market brands of levofloxacin tablet from Bangladesh were randomly selected and analyzed using the newly developed and validated method. 3 mL of  $L_S$  was diluted to 10 mL to obtain 6  $\mu\text{g}/\text{mL}$  levofloxacin reference standard solution. Sample solutions of each brand (6  $\mu\text{g}/\text{mL}$ ) were also prepared and assayed for content of levofloxacin against the reference standard. The content of levofloxacin in the marketed brands was determined using

$$\text{Content of levofloxacin(\%)} \text{ per tablet} = \frac{A_s}{A_{st}} \times \frac{W_{st} \times 5 \times 3}{100 \times 50 \times 10} \times \frac{100 \times 50 \times 10}{W_s \times 5 \times 3} \times W \times \frac{P}{100} \times CF$$

**Table 1** Intra-day and inter-day precision determined for three different concentrations of levofloxacin ( $n=3$ ).

Concentration ( $\mu\text{g}/\text{mL}$ )	Intra-day precision			Inter-day precision		
	Absorbance measured (Mean $\pm$ SD)	RSD (%)	Average potency (%)	Absorbance measured (Mean $\pm$ SD)	RSD (%)	Average potency (%)
4	0.4113 $\pm$ 0.0006	0.140	98.96	0.4110 $\pm$ 0.0010	0.240	98.96
6	0.6147 $\pm$ 0.0006	0.094	98.60	0.6153 $\pm$ 0.0006	0.094	98.70
8	0.8210 $\pm$ 0.0010	0.122	98.77	0.8213 $\pm$ 0.0006	0.070	98.81

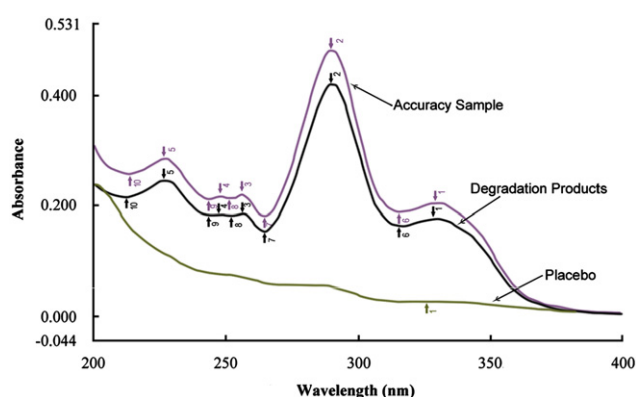
where  $A_s$  is the absorbance of generic sample solution,  $A_{st}$  is the absorbance of levofloxacin reference standard solution,  $W_s$  is the weight of generic sample powder (mg),  $W_{st}$  is the weight of levofloxacin reference standard powder (mg),  $W$  is the average weight of tablet (mg),  $P$  is the potency of standard levofloxacin hemihydrates and  $CF$  is the conversion factor of levofloxacin hemihydrate to levofloxacin (0.976).

**Table 2** Short term stability determined by the proposed method ( $n=3$ ).

Conc. declared ( $\mu\text{g}/\text{mL}$ )	Conc. found (mean $\pm$ SD, $\mu\text{g}/\text{mL}$ )	RSD (%)	Average potency (%)
2	1.963 $\pm$ 0.006	0.295	98.19
4	3.957 $\pm$ 0.006	0.146	98.91
6	5.963 $\pm$ 0.006	0.097	99.41

**Table 3** Recovery/accuracy for five different concentrations of levofloxacin by the proposed method.

Dosage form	Label claim	Amount added (%)	Recovery (%)
Pre formulated granules	500 mg	80	99.05
		90	100.07
		100	99.43
		110	99.00
		120	99.66



**Figure 1** Specificity of the method determined by comparing the spectra of accuracy sample, placebo and degradation products.

### 3. Results and discussion

#### 3.1. Method development and optimization

Levofloxacin is almost insoluble in aqueous medium and freely soluble in organic solvents like methanol and acetonitrile. During the development phase, the use of a few milliliters of acetonitrile and methanol with water as the diluent resulted in preferable outcome in UV analysis. The solvent composition was optimized to water (9):methanol (0.5):acetonitrile (0.5). The pre-determined wavelength of maximum absorption ( $\lambda_{\text{max}}$ ) was 292 nm.

#### 3.2. Method validation

##### 3.2.1. Linearity and range

The calibration curve obtained was evaluated by its correlation coefficient. The absorbance of the samples in the range of 1.0–12.0  $\mu\text{g}/\text{mL}$  was linear with a correlation coefficient ( $R^2$ ) greater than 0.999. The LOD and LOQ were calculated as 0.021  $\mu\text{g}/\text{mL}$  and 0.064  $\mu\text{g}/\text{mL}$  respectively.

##### 3.2.2. Intra-day and inter-day precision

The intra-day and inter-day precision study (Table 1) of the developed method confirmed adequate sample stability and method reliability where all the RSDs were  $<2\%$ .

##### 3.2.3. Stability

Stability study's results were within the acceptance range (Table 2) and indicated the samples stability over 24 h (short-term).

##### 3.2.4. Accuracy/recovery

Results within the range of 99.00–100.07% ensure an accurate method (Table 3) as well as indicate non-interference with the excipients of formulation.

##### 3.2.5. Specificity in the presence of excipients

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 (Fig. 1).

##### 3.2.6. Content of levofloxacin in marketed brands

Levofloxacin content of five marketed products determined by the proposed method (Table 4) was in good agreement with the label claims and was in the range of 98.05–99.47% with the RSD values of 0.067–0.140% respectively.

**Table 4** Content of levofloxacin in five marketed products determined by the proposed method.

Brand	Label claim (mg)	Amount found (mean $\pm$ SD, mg)	Potency (%)	RSD (%)
Brand 1	500	495.2 $\pm$ 0.7	99.05	0.140
Brand 2	500	491.4 $\pm$ 0.5	98.05	0.094
Brand 3	500	492.7 $\pm$ 0.5	98.54	0.094
Brand 4	250	248.0 $\pm$ 0.2	99.19	0.067
Brand 5	250	248.7 $\pm$ 0.3	99.47	0.128

#### 4. Conclusion

The results and the statistical parameters demonstrate that the proposed UV spectrophotometric method is simple, rapid, specific, accurate and precise. Therefore, this method can be used for the determination of levofloxacin either in bulk or in the dosage formulations without interference with commonly used excipients and related substances.

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