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### SPECTROPHOTOMETRIC METHOD FOR DEGRADATION STUDY OF LEVOFLOXACIN

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#### ABSTRACT

Spectrophotometric method for degradation study of Levofloxacin was described. The ultra violet spectrum of acidic, basic and oxidative degraded product was found to substantial difference from pure drug. The extent of degradation can be calculated by comparing the decrease in absorbance at selective wavelength. The UV spectrum of Levofloxacin showed maximum absorbance at 287nm. The solution in alkaline, acidic and oxidative condition showed a decrease in absorbance at 287nm. So the decrease in absorbance at 287nm was used as measure of extent of degradation in all the degradation conditions. The degradation in acidic condition was found to be more when compared to alkaline and oxidative conditions.

#### KEYWORDS

Levofloxacin, UV Spectrophotometry and Degradation.

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#### 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<sup>1</sup>. The addition of 6-fluoro and 7-piper-azinyl 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<sup>2-4</sup>. Chemically Levofloxacin is (S)-9-fluoro-2, 3-dihydro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-7H-pyrido [1,2,3-de]-1,4-benzoxazine-6-carboxylic acid. It has a molecular formula of C<sub>18</sub>H<sub>20</sub>FN<sub>3</sub>O<sub>4</sub> and its structure was given in Figure No.1.

Several HPLC assay methods have been reported for the determination of Levofloxacin or its stereoisomers<sup>5-9</sup>. Literature survey revealed that various analytical methods such as high performance thin layer chromatography (HPTLC)<sup>10</sup> and conductometry<sup>11</sup> have been reported for the estimation of Levofloxacin. Recently some UV spectrophotometric methods were also reported for estimating Levofloxacin using various solvents like 0.1 M hydrochloric acid<sup>12</sup>, 100% methanol<sup>13</sup> or acetonitrile<sup>14</sup> (Figure No.2). 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)<sup>15</sup> and demonstrated excellent specificity, linearity, precision and accuracy for Levofloxacin.

## Materials and Methods

### Drug and Chemicals

All chemicals used were of A.R Grade from S.D. Fine – Chem, Merck, Fischer scientific, and spectrochem, Mumbai. Authentic drug sample of Levofloxacin was obtained as a gift sample by Hetero drugs Ltd., Hyderabad.

### Instrument

Labindia – 3000+ UV / Vis double beam Spectrophotometer with a fixed slit width (2 nm) and 10 millimeter quartz cell was used to obtain spectrum and absorbance measurement.

### Preparation of stock solution

Standard stock solution was prepared by dissolving accurately weighed 100mg of Levofloxacin in water and the volume was made up to 100ml with water in

a 100ml volumetric flask (1000 mcg/ml). 10ml of above stock solution was diluted to 100ml with water (100mcg/ml). 1ml of stock solution was taken in 10ml standard flask diluted to 10ml with water to get the concentration 10mcg/ml. The absorbance of resulting solution was measured against respective blank solution in the UV region of 200-400nm, which shows maximum absorbance at 287nm.

### Forced degradation Study

#### Alkaline Degradation Study

#### Alkaline degradation was done against 0.1N NaOH

##### Procedure

Accurately weighed 10mg of Levofloxacin was dissolved in 10ml volumetric flask with 100ml water. From the above stock solution, 1ml is taken and is made up to 10ml with 0.1N NaOH in 10ml volumetric flask. From that solution, 1ml is taken and made up to 10ml in 10ml volumetric flask at time intervals of 1hr, 2hr, 24hr and 48hrs (Table No.1 and Figure No.3).

#### Acidic Degradation study

#### Acidic degradation was done against 0.1N Hcl

##### Procedure

Accurately weighed 100mg of Levofloxacin was dissolved in 100ml of water in 100ml of volumetric flask. From the above solution 1ml is taken out and it is made up with 0.1N Hcl in 10ml volumetric flask. From the solution 1ml is taken and made up to 10ml volumetric flask at time intervals of 1hr, 2hr, 24hr and 48hrs (Table No.2 and Figure No.4).

#### Oxidative Degradation

#### Oxidative degradation was done against 0.3% H<sub>2</sub>O<sub>2</sub>

##### Procedure

Accurately weighed 100mg of Levofloxacin was dissolved in 100ml of water in 100ml of volumetric flask. From that solution 1ml is taken out and it is taken out and it is making up to 10ml with 0.3% H<sub>2</sub>O<sub>2</sub> in 10ml volumetric flask. From that solution 1ml is taken and made up to 10ml in 10ml volumetric flask at time intervals of 1hr, 2hr, 24hr and 48hr. The absorbance of above solutions were noted (Table No.3 and Figure No.5).

## RESULTS AND DISCUSSION

Levofloxacin showed maximum absorbance at 287nm. The sample in NaOH showed decrease in the absorbance at 287nm and was used as the measure of extent of degradation in NaOH. In case of acidic condition, decrease of absorbance is seen due to

degradation but in comparison to alkaline condition more. Absorbance is seen at 287nm and there is no other peak observed. The overlain zero order spectrum of Levofloxacin, NaOH degraded and Hcl degraded respectively showed in Figure No.2.

**Table No.1: Degradation of Levofloxacin in alkali condition [0.1N NaOH]**

S.No	Time	Absorbance
1	0 hrs	0.590
2	2 hrs	0.554
3	48 hrs	0.524

**Table No.2: Degradation of Levofloxacin in acidic condition [0.1N Hcl]**

S.No	Time	Absorbance
1	0 hrs	0.735
2	2 hrs	0.710
3	48 hrs	0.580

**Table No.3: Degradation of Levofloxacin in oxidative stress condition (0.3% H<sub>2</sub>O<sub>2</sub>)**

S.No	Time	Absorbance
1	0 hrs	0.643
2	2 hrs	0.457
3	48 hrs	0.363

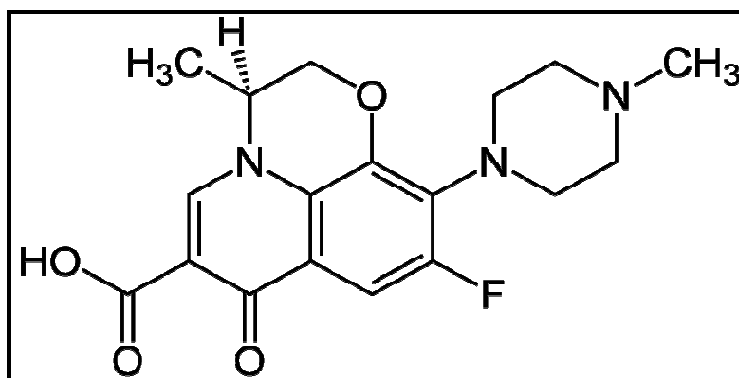


Figure No.1: Structure of Levofloxacin

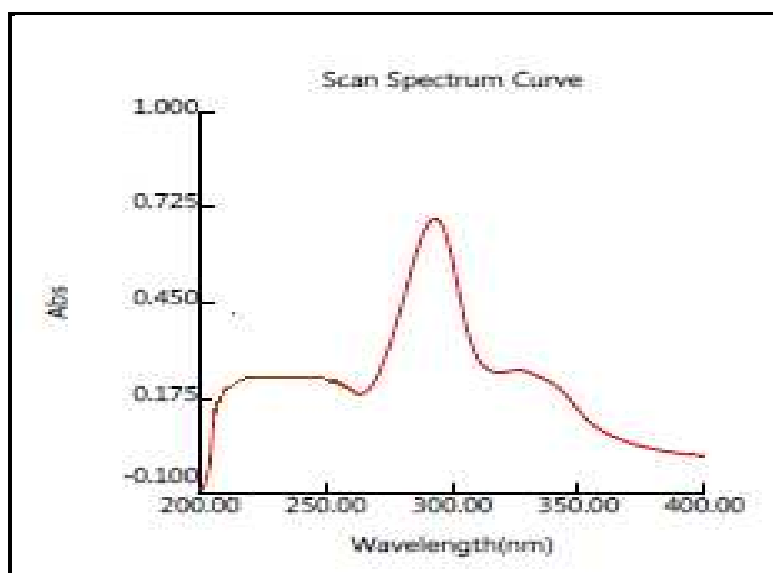


Figure No.2: Spectra of Levofloxacin at 287nm

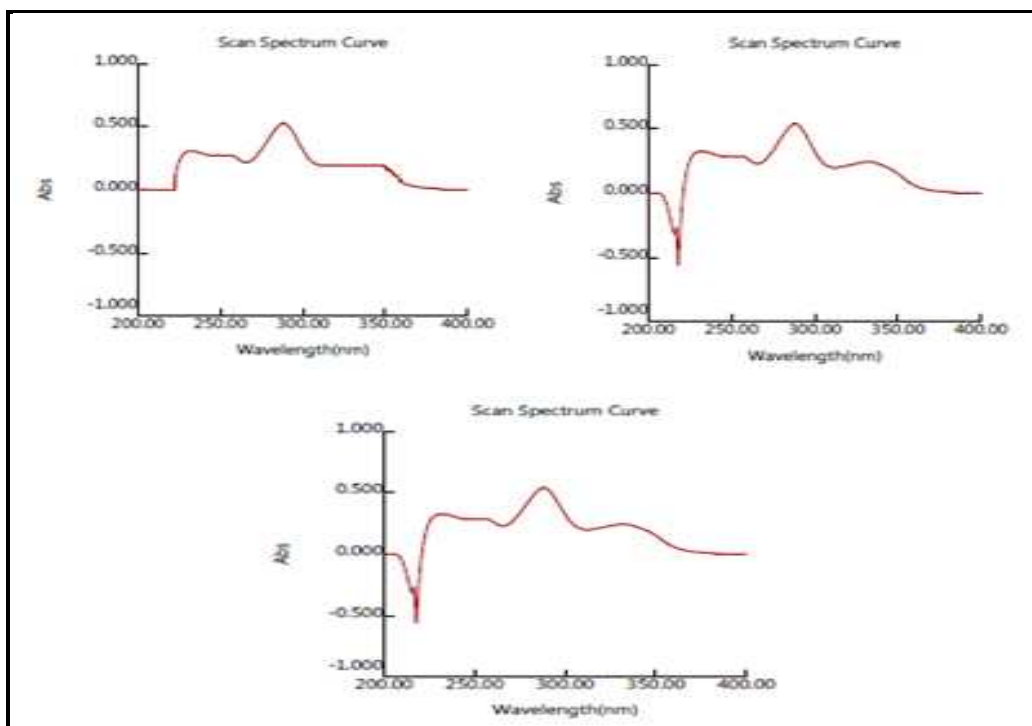


Figure No.3: Degradation of Levofloxacin in alkali condition [0.1N NaOH]

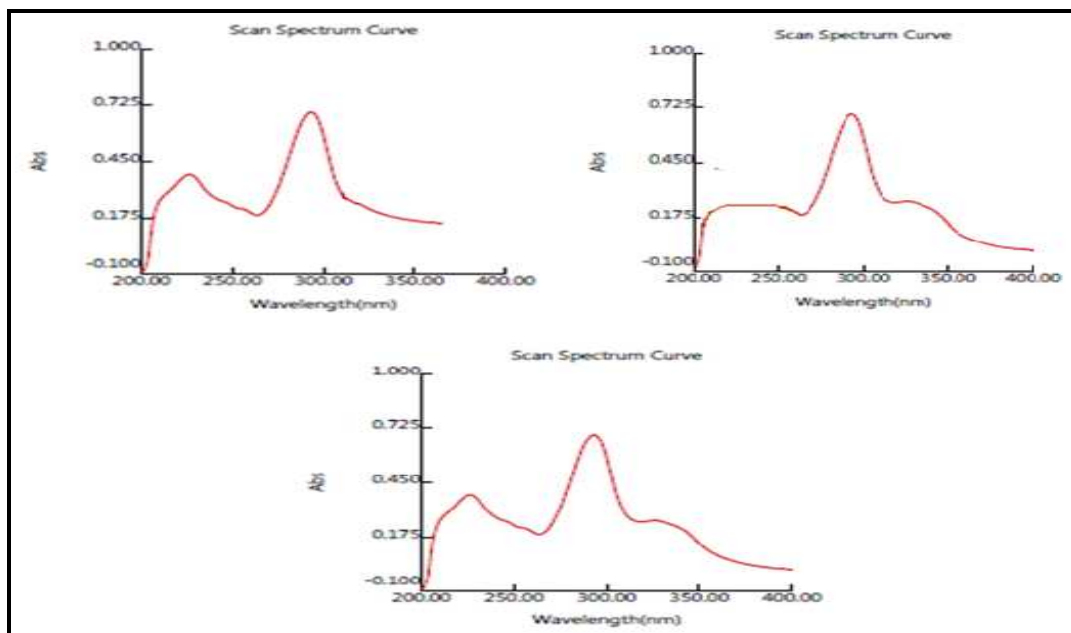


Figure No.4: Degradation of Levofloxacin in acidic condition [0.1N HCl]

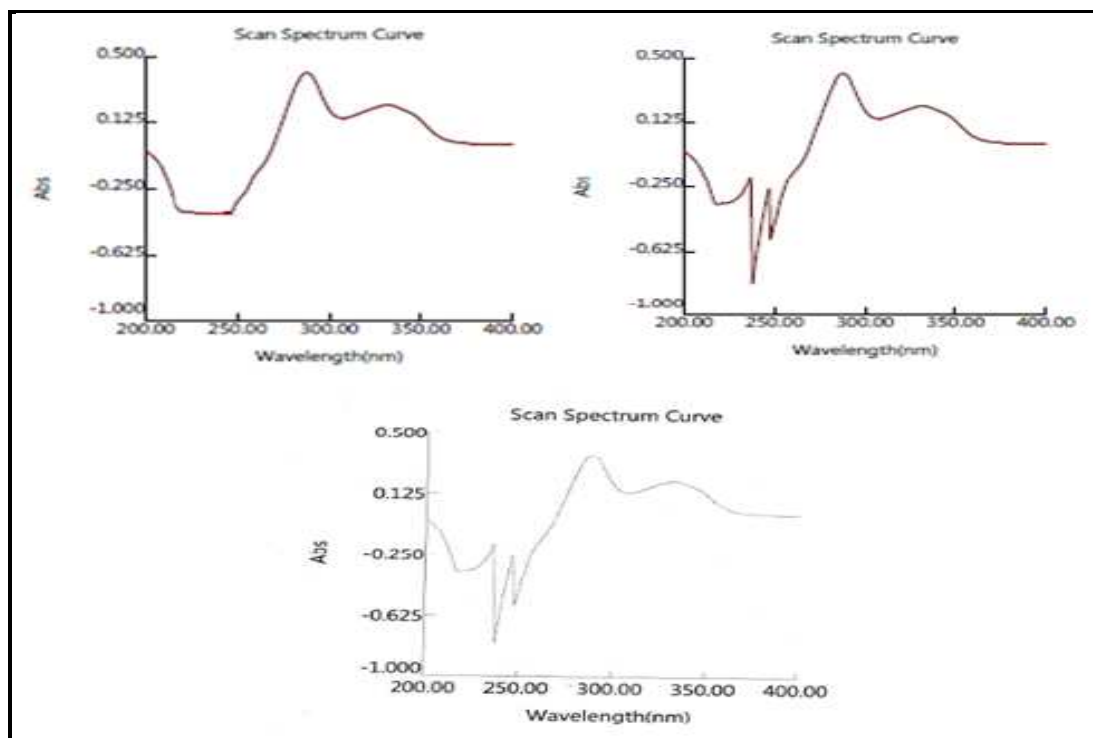


Figure No.5: Degradation of Levofloxacin in oxidative stress condition (0.3% H<sub>2</sub>O<sub>2</sub>)

## CONCLUSION

The ultra violet spectrum of both acidic and basic degraded products was found to be substantially different from pure drug. The extent of degradation can be calculated by comparing the decrease in absorbance at selective wavelength. The solution which was degraded in NaOH, HCl and oxidative stress conditions showed a decrease in the absorbance at 287nm and was measured as an extent of degradation. From the degradation study, it is found that Levofloxacin is more sensitive to acidic condition.

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