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### QUANTITATIVE DETERMINATION OF LEVOFLOXACIN HEMIHYDRATE IN BULK AND TABLETS BY UV SPECTROPHOTOMETRY, ZERO AND FIRST ORDER DERIVATIVE METHODS

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#### ABSTRACT

Two Simple, rapid, accurate and economical UV Spectrophotometry, Zero and First Order Derivative methods have been developed for the determination of levofloxacin hemihydrate in bulk and tablets. In Distilled water the  $\lambda_{max}$  of the drug was found to be 288nm. The same spectrum was derivative into zero, first order derivative, using UV probe software of instrument (Shimadzu-1800). The amplitude of the trough was recorded at 300 nm. In both proposed methods, levofloxacin hemihydrate follows linearity in the concentration range 2 - 10  $\mu\text{g/ml}$  with a correlation coefficient of 0.9999. Assay results were in good agreement with label claim. The methods were validated statistically and by recovery studies. The relative standard deviations were found to be less than 2% with excellent precision and accuracy.

#### KEYWORDS

Levofloxacin hemihydrate, UV-spectrophotometry, Zero order derivative, First order derivative method. Anti-diabetic, Synergistic effect, *Coccinia grandis Voigt* and *Trigonella foenum-graecum*.

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#### INTRODUCTION

Levofloxacin hemihydrate (Figure No.1), (-)-(S)-9-fluro-2, 3-dihydro-3- methyl-10-(4-methyl-1-piperziny)-7-oxo-7H pyrido [1, 2, 3-de]-1, 4-benzoxazine-6-caboxylic acid hemihydrates (Figure No.1) is broad spectrum fluorinated quinolone antibacterial (The Merck Index, 2001). Levofloxacin comes under category quinolones. It acts by inhibiting bacterial DNA gyrase (topoisomerase II) and topoisomerase IV enzymes which are required for DNA replication transcription, repair and recombination<sup>1</sup>. And thus causes bacterial lyses

(Goodman and Gillman, 2001). Levofloxacin prepared as hemihydrate, whose molecular mass is  $369.93 \text{ g mol}^{-1}$ , is presented as white to light yellow needlelike crystals, that melt at approximately  $226^\circ\text{C}$ . Its solubility is nearly constant from pH 0.6 to 5.8 ( $100.0 \text{ mg mL}^{-1}$ ). Above pH 5.8, solubility increases sharply, reaching a maximum of  $272 \text{ mg mL}^{-1}$  at pH 6.7, beyond which it decreases to a minimum of  $50.0 \text{ mg mL}^{-1}$ .<sup>2</sup> In literature, various analytical methods such as HPLC (Bottcher *et al.*, 2001; Hairui *et al.*, 2002; Wong *et al.*, 2001), HPTLC (Meyyanathan *et al.*, 2003) and conductometry (Altioka and Atkosar, 2002) have been reported for estimation of levofloxacin.

Most spectrophotometric methods in the literature for analysis of levofloxacin is based on the formation of ion-complexes,<sup>3,6</sup> which use dye as Eriochrome black,<sup>5</sup> bromophenol blue, bromocresol green,<sup>3,5</sup> eosin, merbromin<sup>4</sup> and chromogenic reagent such as Folin-Ciocalteau<sup>5</sup>. The addition of these substances usually increases the cost of analysis and sample preparation is time consuming. Besides cost, toxicity of reagents and solvents used in the analysis should also be considered. Exposure to merbromin even at low concentrations and short exposure time can cause poisoning. The complexes formed normally need extraction with organic solvents, for example, chloroform,<sup>3,5</sup> which in addition to further increase the cost of analysis and require safe handling and proper disposal. In addition, there are no official methods for determination of this active substance<sup>3,8</sup>.

Thus, the aim of this study was to develop and validate a fast, simple and cost-effective UV-spectrophotometric alternative method and Zero and first order derivative methods for analysis of both active pharmaceutical ingredient and commercial formulation of levofloxacin.

The objective of the present work is to develop simple, rapid and economical UV spectrophotometry, Zero and First Order Derivative methods for determination of levofloxacin hemihydrates in bulk and finished products.

## MATERIALS AND METHODS

### Materials

LFX was received as a gift from Arabindo laboratories, Hyderabad, India. Distilled water was used as solvent for all analysis. A Shimadzu spectrophotometer model number 1800 was used for the analysis.

### Preparation of standard stock solution and study of calibration curves

Standard stock solution containing  $100 \text{ }\mu\text{g/ml}$  of levofloxacin hemihydrate was prepared in (5% v/v) acetonitrile. Different aliquots were taken from the stock, diluted to 10 ml mark with the same solvent to obtain  $2 \text{ }\mu\text{g/ml}$ ,  $3 \text{ }\mu\text{g/ml}$ ,  $4 \text{ }\mu\text{g/ml}$ ,  $5 \text{ }\mu\text{g/ml}$ ,  $6 \text{ }\mu\text{g/ml}$ ,  $7 \text{ }\mu\text{g/ml}$ ,  $8 \text{ }\mu\text{g/ml}$ ,  $9 \text{ }\mu\text{g/ml}$ ,  $10 \text{ }\mu\text{g/ml}$  concentrations. The solutions were scanned on UV-visible spectrophotometer (Shimadzu-2450 with UV probe 2.31 software in the UV range 200 - 400 nm. Levofloxacin hemihydrate showed absorbance maxima at 288 nm. The same spectra were derivative into Zero and first order derivative, using UV probe software of instrument. The amplitudes of the corresponding troughs were measured at 300 nm Figure No.2 - 4. In both the methods, levofloxacin hemihydrate follows linearity in the concentration range 1 - 10  $\mu\text{g/ml}$ . Figure No.5 and 6.

### Preparation of sample solution

For analysis of commercial formulation, average weight of twenty tablets were taken and crushed to a fine powder. An accurately weighed quantity of powder equivalent to 100 mg of levofloxacin was transferred into 100 ml volumetric flask containing 25 ml distilled water, shaken manually for 10 min, volume was adjusted to mark with same solvent and filtered through Whatmann filter paper no.41. After appropriate dilution absorbance was recorded at 288 nm and amplitude of the trough (first order derivative) was recorded at 300 nm. The results are shown in Table No.1.

### Recovery studies

The recovery studies were carried out at three different levels i.e. 50%, 100% and 150% level both in UV spectrophotometry and first order derivative spectrophotometry method. To the reanalyzed sample solution, a known amount of standard drug

solution was added and reanalyzed by the proposed methods. Figure No.7 and 8. The results are shown in Table No.2.

**Precision**

Intra-day and inter-day repeatability are the two methods for the evaluation of responses of sample solutions of precision for the proposed method. All solutions were prepared freshly and precision is expressed as relative standard deviation (R.S.D.) amongst responses in each case. Variation among interday and intra-day was taken to determine intermediate precision of the proposed methods. Different levels (low, medium, high) of drug

concentrations in triplicates were prepared three different times in a day and studied for intra-day variation. Same protocol was followed for three different days to study inter-day variation. Percent RSD (% RSD) was found to be lower than 2% in each level (Table No.2).

**Ruggedness**

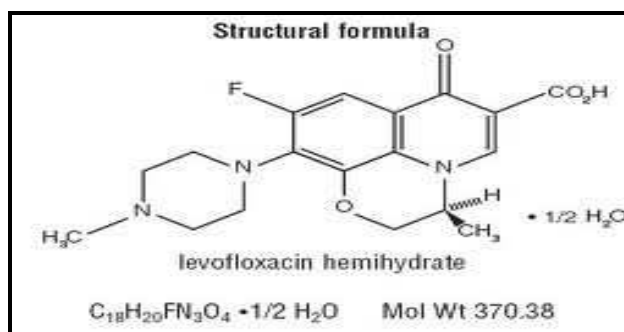
Ruggedness of the proposed methods was determined by analyzing aliquots from homogenous slot by different analyst using similar operational and environmental conditions and data is presented in Table No.2.

**Table No.1: Results of assay**

S.No	Drug	UV-Spectrophotometry		First order derivative	
		Amount found (%) [ n = 6]	% RSD	Amount found (%) [ n = 6]	% RSD
1	Levofloxacin (500mg/tablet)	99.59	0.419	100.46	0.453

**Table No.2: Summary of Validation Parameters**

S.No	Parameters	UV spectro photometry	First order derivative spectrophotometry
1	% Recovery ( n = 9)	99.99	100.16
2	% RSD	0.27	0.757
	<b>Precision [% RSD]</b>		
3	Intra-day ( n = 3)	0.879 -1.123	0.614 -1.757
4	Inter-day ( n = 3)	0.320 - 1.357	0.435 - 0.749
5	Repeatability (% RSD, n = 6)	0.631	0.257
	<b>Ruggedness [% RSD]</b>		
6	Analyst-I ( n = 6)	1.126	0.321
7	Analyst -II(n=6)	1.015	0.554



**Figure No.1: Structure of Levofloxacin Hemihydrate**

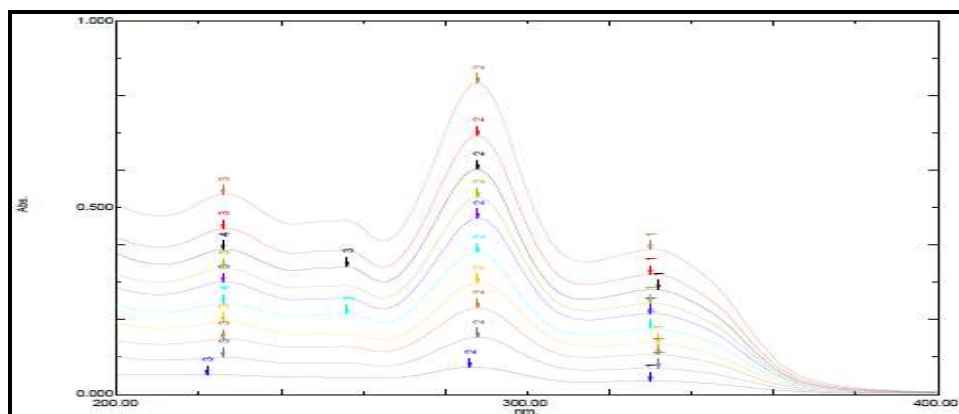


Figure No.2: An Overlain Spectrum of Levofloxacin Hemihydrate in distilled water

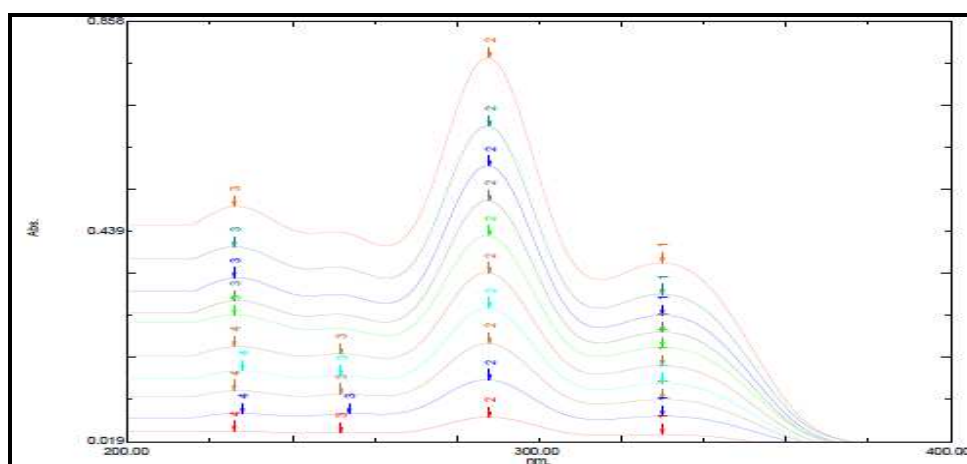


Figure No.3: An Overlain Zero Order Derivative Spectrum of Levofloxacin Hemihydrate in Distilled water

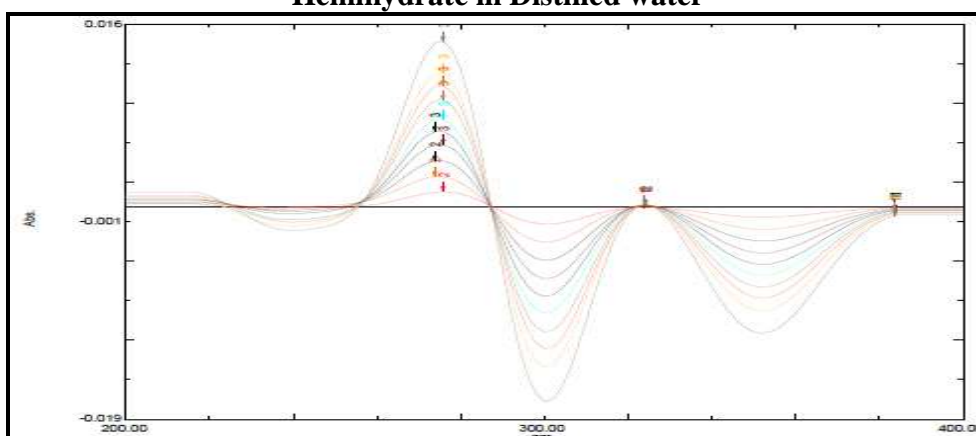


Figure No.4: An Overlain First Order Derivative Spectrum of Levofloxacin Hemihydrate in Distilled water

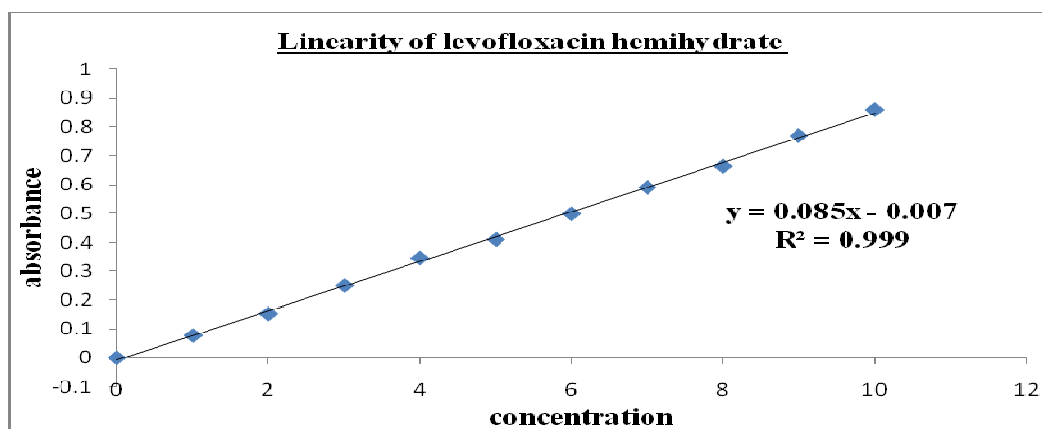


Figure No.5: Linearity of Levofloxacin Hemihydrate UV Spectrophotometry

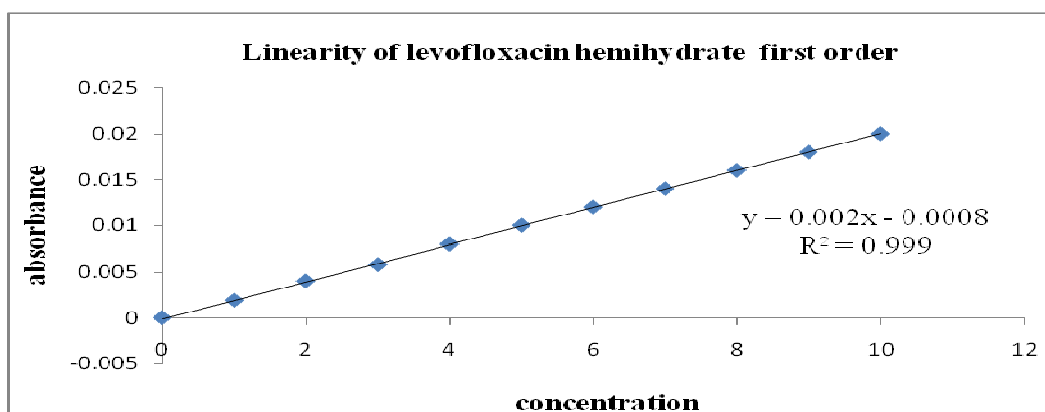


Figure No.6: Linearity of Levofloxacin Hemihydrate (First Order Derivative Spectrophotometric Method)

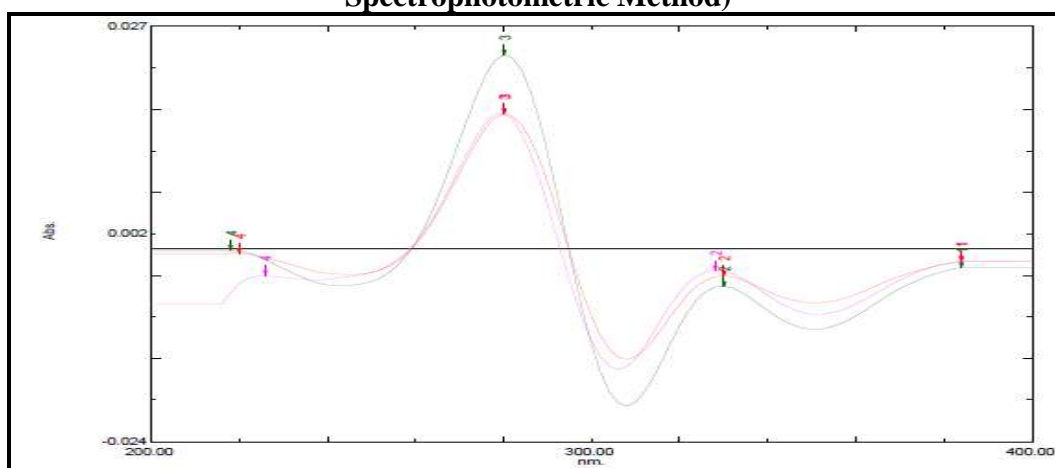
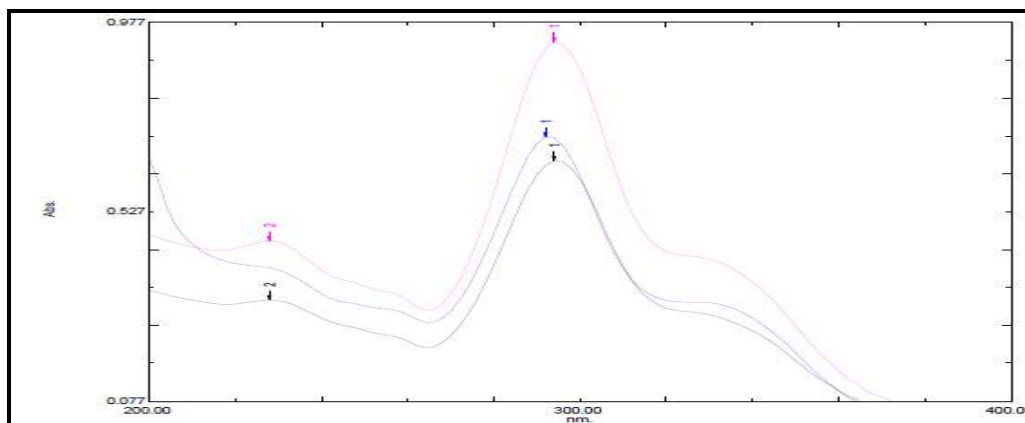


Figure No.7: Recovery Studies of Levofloxacin Hemihydrates 50%, 100%, 150% (First Order Derivative)



**Figure No.8: Recovery Studies of Levofloxacin Hemihydrate 50%, 100%, 150% UV Spectrophotometry**

## RESULTS AND DISCUSSION

Levofloxacin hemihydrate showed absorbance maximum at 288 nm in distilled water. In first order derivative spectrum, 300 nm is the amplitude of the trough was recorded. In both the methods, 2 - 10 µg/ml is the linearity followed by the levofloxacin hemihydrate showing linear regression equations  $Y = 0.085 X + 0.0032$  in UV-spectrophotometry method ( $r^2 = 0.9999$ ) and  $Y = 0.002 X + 0.0008$  for first order derivative method ( $r^2 = 0.9999$ ).

By the proposed methods the amount of drug determined was in good agreement with the label claimed. These methods were validated for accuracy, precision and ruggedness as per USP (USP, 2005). The results are as shown in Table No.2. The results of validation parameters demonstrated that the procedure is accurate, precise and reproducible (relative standard deviation < 2%). Both these proposed methods are simple, economical, rapid and can suitably be used for the determination of levofloxacin in tablet formulation.

## CONCLUSION

The solvent used in this method for the analysis of levofloxacin hemihydrate in both bulk and formulation was distilled water so it was very economical. And also this derivative spectroscopic method was useful for accurate determination of absorption maxima. Both these proposed methods are simple, economical, rapid and can suitably be used for the routine analysis and quantitative

determination of levofloxacin hemihydrate both in active pharmaceutical ingredient and in tablet formulation.

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