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# DEVELOPMENT AND VALIDATION OF DIFFERENTIAL SPECTROPHOTOMETRIC METHOD FOR ASSAY OF ENROFLOXACIN IN BULK AND TABLET FORMULATION

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**Abstract:**

*A simple, precise, and accurate spectrophotometric method has been developed and validated for the estimation of Enrofloxacin in bulk as well as tablet formulation. The UV spectra of Enrofloxacin were obtained in 0.1N HCl and 0.1N NaOH, and the overlain spectra showed maximum absorbance at 271.0 nm (maxima) and 276.5 nm (minima) in 0.1N NaOH and HCl respectively. The drug follows linearity in the range of 5-30 µg/ml ($R^2$= 0.988). Both intra- and inter-day precision showed % RSD< 2 while LOD, and LOQ were 0.320 and 0.971 respectively. The method was validated as per ICH guidelines.*

***Keywords**: Enrofloxacin, Differential UV Spectrophotometry, Validation, ICH, Enrofloxacin Tablet Analysis, etc.*

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## 1. INTRODUCTION:

Enrofloxacin or [1-cyclopropyl-6-fluoro-7-(4-ethyl-1-piperazinyl)-1,4-dihydro-4-oxo-3 quinoline carboxylic acid][1]. The chemical structure of the drug is given in (fig. 1). It is a synthetic antibacterial agent from the class of the fluroquinolone carboxylic acid derivatives. It has antibacterial activity against a broad spectrum of Gram negative and Gram positive bacteria. Enrofloxacin is used for the treatment of individual pets and domestic animals.

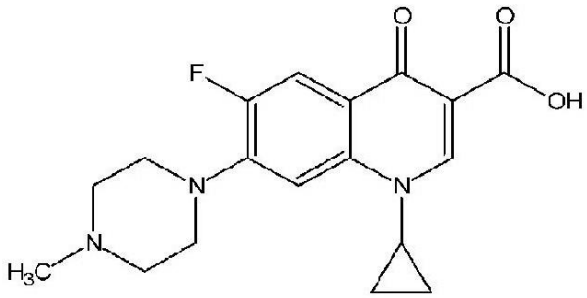


**Fig. 1: Chemical Structure of Enrofloxacin**

Literature survey shows that several analytical methods for the determination of Enrofloxacin include RP-HPLC[2, 3], HPLC[4-6], RP-LC[7], LC[8] and dissolution study[9] have been reported. Many pharmaceutical products have been assayed by this method[10-13]. But Differential UV spectrophotometric[14] method of Enrofloxacin was not reported so far. So an attempt was made to develop a novel, simple, accurate spectrophotometric method for the determination of Enrofloxacin in tablet formulation.

## 2. MATERIALS AND METHODS:

A SHIMADZU 1650 double beam UV/ Visible Spectrophotometric (Tokyo, Japan) with 10mm quartz cuvettes were used for spectral measurements. A calibrated Electronic Balance was used for weighing the reagents. Digitalis Ultrasonic Cleaner was also used during the analysis.

Pure drug Enrofloxacin was obtained as a gift sample from Alembic Pharmaceutical Pvt. Ltd. (Baroda, Gujarat). Ethanol, Hydrochloric acid, and Sodium Hydroxide pellet LR (Purified) were purchased from Research Lab Fine Chem Pvt. Ltd., Mumbai, India. Analytical grade distilled water, Baytril (150mg) was purchased from a local market.

### 2.1 Preparation of standard stock solution:

Standard stock solution for the drug was prepared by dissolution accurately weighed 10 mg of pure Enrofloxacin in ethanol up to 10 ml. This gave a stock solution of 1000 µg/ml. From this, 1 ml of the solution were taken into 2 separate 10 ml volumetric flasks and were diluted up to the mark with 0.1N NaOH and 0.1N HCl to produce working standard solutions of concentration 100 µg/ml [15].

### 2.2 Preparation of Calibration Curve:

Different aliquots (0.5, 1.0, 1.5, 20, 25, 30 ml) were taken from the respective working standard solutions in separate 10 ml volumetric flasks and finally diluted up to the mark with 0.1N HCl and 0.1N NaOH solutions to prepare a series of concentration ranging from 5-30 µg/ml as a reference and test solutions. The difference spectrum for Enrofloxacin was recorded by placing the drug in 0.1N NaOH in a reference cell and 0.1N HCl in the sample cell [16]. The difference in absorbance (Table 1) between 271.0 nm (maxima) and 276.5 nm (minima) was calculated to find out the amplitude. A Calibration curve was plotted by taking the concentration of the drug (µg/ml) on X-axis and amplitude on Y-axis. The overlain difference UV absorption spectrum of Enrofloxacin concentration of 20 µg/ml in both acidic and basic media is shown in (fig. 2).

### 2.3 Assay of Tablet Formulation:

Twenty tablets were weighed accurately and powdered finely. A quantity of tablet powder equivalent to 10 mg of Enrofloxacin was accurately weighed and transferred into in 10 ml volumetric flask, 5 ml of ethanol was added and the content was ultrasonicated for 20 min; the volume was made up to the mark and mixed well [17]. The solution was further filtered by using Whatman filter paper to remove any unwanted particulate matter. The filtered solution was further appropriately diluted with 0.1N NaOH and 0.1N HCl separately for analysis as already described. The amount of drug present in the sample was determined using the calibration curve of the standard drug.

### 2.4 Method validation:

The proposed method was validated as per ICH Q2 (R1) guidelines for linearity, accuracy, precision, LOD, and LOQ [23].

To check the accuracy of the proposed method, recovery studies were carried out at 50, 100, and 150% of the test concentration. The recovery study was performed three at each level [18]. The intra-day and inter-day precision of the method was determined by careful determination of six replicates of a fixed concentration of the drug within the Beer's range and finding out the amplitude by the method. The % RSD was calculated [19].

LOD and LOQ of the drug were calculated by using calibration standard [20].

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where σ = S.D of response

S = slope of the regression equation

## 3. RESULTS AND DISCUSSION:

The proposed method is based on a differential spectrophotometric estimation of Enrofloxacin in Bulk and Tablet formulation in UV region by using ethanol as solvent. The values are expressed as amplitude i.e. the difference between two equimolar solutions of the analyte in two different chemical forms exhibiting different spectral properties as shown in (fig 3). The difference spectrum of Enrofloxacin in 0.1N HCl was recorded by taking 0.1N NaOH solution as a reference. The difference spectral analysis shows the maximum absorbance at 271.0 nm and minimum absorbance at 276.5 nm.

The validation parameters are shown in (Table 2). Beer's law was obeyed in the concentration range of 5 to 30 µg/ml. The accuracy of the proposed method was evaluated by % Recovery studies of the drug. The average recovery ranged from 98.36-102.3% as shown in (Table 3). The assay result of the commercial formulation was found to be 100.01% as shown in (Table 4). Similarly, the % RSD for Intraday and Interday Assay were found to be 0.43 and 0.44 respectively. The values of LOD and LOQ were found to be 0.320 and 0.971 µg/ml which indicated good sensitivity of the proposed method.

**TABLE 1: RESULTS OF STANDARD CALIBRATION**

| Concentration (µg/ml) | Absorbance in 0.1N HCl | Absaorbance in 0.1N NaOH | Amplitude |
|---|---|---|---|
| 5 | 0.2132 | 0.0247 | 0.1885 |
| 10 | 0.3784 | 0.0352 | 0.3432 |
| 15 | 0.5126 | 0.0487 | 0.4639 |
| 20 | 0.6159 | 0.0601 | 0.5558 |
| 25 | 0.7312 | 0.0701 | 0.6611 |
| 30 | 0.9342 | 0.1061 | 0.8281 |

**TABLE 2: VALIDATION PARAMETERS**

| Parameters | Values |
|---|---|
| λ max (nm) | |
| (0.1N HCl) | 276.5(minima) |
| (0.1N NaOH) | 271.0(maxima) |
| Beer's limit | 5-20 µg/ml |
| Reg. equation | Y= 0.026x + 0.0441 |
| $R^2$ | 0.988 |
| Slope | 0.026 |
| Intercept | 0.0441 |
| Precision (%RSD) | |
| Intraday | 0.43 |
| Interday | 0.44 |
| Accuracy | 98.36-102.3 |
| LOD (µg/ml) | 0.320 |
| LOQ(µg/ml) | 0.971 |
| Linearity | 5-30 |

**TABLE 3: RESULTS OF RECOVERY STUDIES**

| Amount of Standard spiked(µg/ml) | % Standard Recorded | %RSD |
|---|---|---|
| 50% | 98.36 ± 0.133 | 0.13 |
| 100% | 102.3 ± 0.017 | 0.04 |
| 150% | 99.38 ± 0.011 | 0.02 |

**TABLE 4: ANALYSIS OF COMMERCIAL FORMULATION OF ENROFLOXACIN**

| Brand Name | Label Claim (mg) | %Recovery ± SD |
|---|---|---|
| Baytril | 150 | 100.01 ± 0.79 |

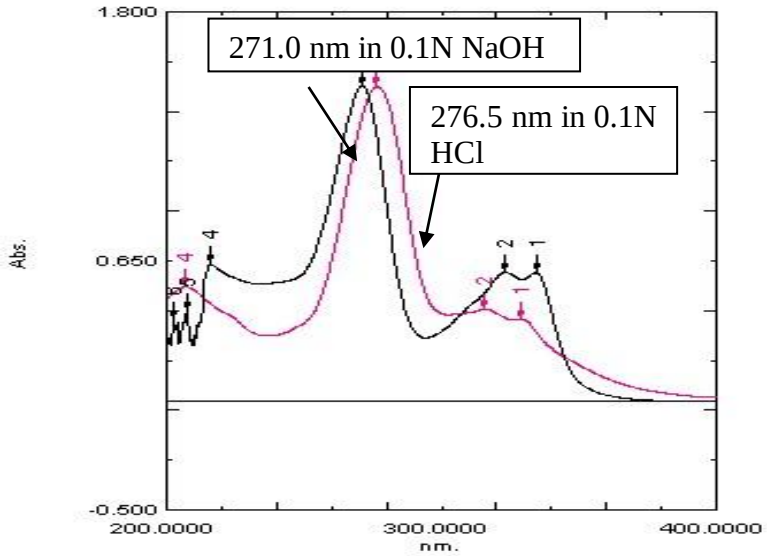


**Fig. 2: Overlain Spectra of Enrofloxacin in 0.1N HCl and 0.1N NaOH**

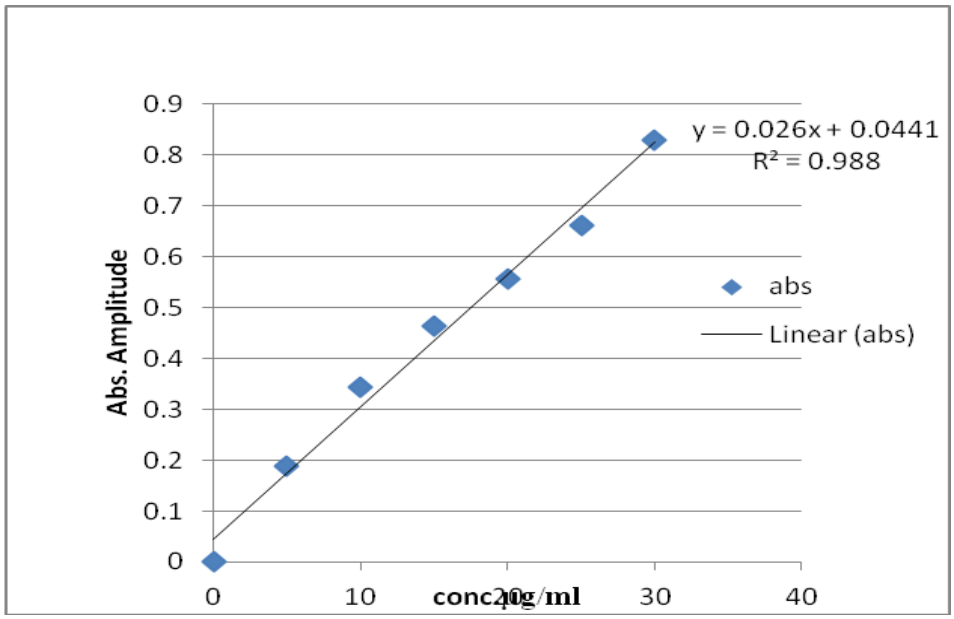


**Fig. 3: Standard Calibration Curve (Difference Spectrum) of Enrofloxacin**

## 4. CONCLUSION:

This Differential spectrophotometric technique was quite simple, accurate, precise, reproducible, and sensitive. The Differential spectrophotometric method has been developed for Assay of Enrofloxacin in Bulk and Tablet Formulation. The validation procedure confirms that this is a workable

method for their quantification in the bulk material and also in the tablet formulations.

**5. ACKNOWLEDGEMENTS**

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