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PREFORMULATION STUDIES AND ANALYTICAL METHOD DEVELOPMENT FOR ENROFLOXACIN AND CIPROFLOXACIN: A QUALITY CONTROL APPROACH

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ABSTRACT

Objective: To evaluate the preformulation properties of Enrofloxacin and Ciprofloxacin, including organoleptic, solubility, melting point, pH, FTIR spectra, and linearity, and to establish precise analytical methods for quality control. **Methods:** The drugs were characterized for solubility in various solvents, pH, melting point, and FTIR spectra. Linearity, precision, ruggedness, robustness, and recovery studies were conducted using UV-Vis spectrophotometry. Regression equations and correlation coefficients were determined, and LOD and LOQ values were calculated. **Results:** Enrofloxacin and Ciprofloxacin exhibited distinct solubility profiles and melting points (220°C and 235°C). The pH values were 7.34 and 3.81, respectively. FTIR analysis confirmed characteristic functional groups. Linearity was observed over concentration ranges of 2-10 µg/mL (Enrofloxacin) and 10-50 µg/mL (Ciprofloxacin) with high correlation coefficients (R^2 = 0.9973 and 0.9975). Precision and ruggedness were within acceptable limits, and recovery rates were 98-99.9%. **Conclusion:** The proposed analytical method is simple, precise, and robust, making it suitable for the quality control of Enrofloxacin and Ciprofloxacin in pharmaceutical formulations.

KEYWORDS: Enrofloxacin, Ciprofloxacin, Preformulation, UV-Vis Spectrophotometry, Linearity, FTIR.

INTRODUCTION

Analytical method development and validation are fundamental aspects of pharmaceutical analysis, ensuring reliable and reproducible results for assessing the quality, safety, and efficacy of drug formulations (Lindsay, 2010). Enrofloxacin and ciprofloxacin are broad-spectrum fluoroquinolone antibiotics widely employed for treating bacterial infections in humans and animals, especially in respiratory, urinary tract, and gastrointestinal infections (Martinez et al., 2006). Their presence in pharmaceutical formulations demands precise and accurate analytical methods to confirm the active pharmaceutical ingredient (API) concentration and compliance with regulatory standards.

UV spectroscopy, a rapid, cost-effective, and environmentally friendly analytical technique, has become a preferred method for drug analysis in quality control laboratories (Skoog et al., 2018). This technique involves measuring the absorbance of UV light by the drug molecules, providing quantitative information. Its simplicity, non-destructive nature, and minimal sample preparation requirements make it suitable for routine analysis.

This study emphasizes developing and validating a UV spectroscopy method for the simultaneous estimation of enrofloxacin and ciprofloxacin in marketed formulations. The method follows the International Council for Harmonisation (ICH) guidelines (ICH Q2(R1), 2005) to ensure accuracy, precision, linearity, specificity, and robustness. Accurate quantification is essential to guarantee therapeutic efficacy and prevent adverse effects such as antibiotic resistance due to improper dosing (Ventola, 2015).

By implementing a validated UV spectroscopy method, this research contributes to improved quality control processes, aiding pharmaceutical industries in regulatory compliance and enhancing public health through safe and effective antibiotic formulations.

HPLC methods are commonly employed for their high sensitivity and specificity but involve higher costs and longer analysis times (Shah et al., 2015). **UV spectroscopy**, on the other hand, offers a simpler, faster, and more cost-effective alternative for routine quality control (Pandya et al., 2013).

Studies by **Patel et al. (2012)** demonstrated the use of UV spectroscopy for simultaneous quantification of ciprofloxacin and other drugs, showcasing the method's precision and reliability. Similarly, **Kumar et al. (2016)** validated UV spectrophotometric methods following ICH guidelines, proving their applicability for fluoroquinolones in marketed formulations.

These findings highlight the growing preference for UV spectroscopy in pharmaceutical analysis, especially for its ease of use and compliance with regulatory standards.

MATERIAL AND METHOD

The study required various chemicals, glassware, and instruments. The chemicals used included Enrofloxacin and Ciprofloxacin, both sourced from Sigma Aldrich, along with ethanol, acetone, dimethylformamide, methanol, disodium hydrogen phosphate, potassium dihydrogen phosphate, and sodium chloride, all obtained from Merck. Additionally, n-octanol and dimethyl sulfoxide were procured from CDH, while hydrochloric acid was obtained from Lobachemie. Distilled water of HPLC grade was sourced from Qualikem. These reagents and solvents formed the foundation for the experimental procedures conducted.

Pre-formulation Studies of Enrofloxacin and Ciprofloxacin

Organoleptic Properties

The organoleptic studies of Enrofloxacin and Ciprofloxacin general appearance like colour, odour, appearance and state etc. were performed and observed**.**

Solubility study

An excess amount of the drugs were added to 10 mL volumetric flask individually having different Solvents (i.e Hydrochloric acid (0.1 N), Water, Ethanol, Methanol, Acetone, DMSO and DMF). Drugs were added to this till saturation occurred and shaken at room temperature for 48 h. After that, samples were filtered and analyzed by UV spectroscopy**.**

Determination of Melting Point

For determination of melting point USP method was followed. Small quantities of drugs were placed into a sealed capillary tube separately. The tube was placed in the melting point apparatus. The temperature in the apparatus was gradually increased and the observation of temperature was noted at which Enrofloxacin and Ciprofloxacin started to melt and the temperature when the entire drugs get melted. This method is also known as open capillary

method**.**

pH determination

The determination of pH value of Enrofloxacin and Ciprofloxacin (drugs) solutions were generally performed by pH meter with glass electrode as the indicator electrode and a cell composed of glycury electrode as the reference electrode. The results were noted in triplicate form and average was written against the reference value.

Identification of pure drug

Fourier transform Infrared spectroscopy (FTIR)

FTIR spectroscopy is a widely used technique for investigating materials in the gaseous, liquid or solid phase. It is based on the interaction between electromagnetic radiation and natural vibrations of the chemical bonds among atoms that compose the matter. The major use of infrared spectroscopy is to determine the functional groups of molecules, relevant to both organic and inorganic chemistry.

The Enrofloxacin and Ciprofloxacin (drugs) were mixed separately with 200 mg KBr (FT-IR grade) and pressed into a pellet. The sample pellet was placed into the sample holder and FT-IR spectra were recorded in the range 400-4000 cm-1 in FT-IR spectroscopy**.**

UV-Vis Spectroscopy Method

Selection of solvent of method development

On the basis of solubility study selection of solvent of method development for Enrofloxacin and Ciprofloxacin the solvent were acetone + ethanol 1:1 and water.

Preparation of Standard Stock Solution for Calibration curve

Standard Enrofloxacin stock solution

Accurately weighed Enrofloxacin (10mg) was transferred to a 10ml volumetric flask. Dissolved it in acetone + ethanol 1:1 solution and make up the volume with acetone + ethanol 1:1 then sonicated for five minutes. The resulting solutions contain 1mg/ml of the drug. The stock solution of Enrofloxacin was further diluted with acetone + ethanol 1:1 to obtain the sub- stock in the range of 2-10 µg/ml. acetone + ethanol 1:1solution was used as a blank solution. The resulting solutions were then scanned in UV spectrophotometer from 200 to 400nm**.**

Standard Ciprofloxacin stock solution

Accurately weighed Ciprofloxacin (10mg) was transferred to a 10ml volumetric flask. Dissolved it Water and make up the volume with Water then sonicated for five minutes and the volume was made up with Water. The resulting solutions contain 1mg/ml of the drug. The stock solution of Ciprofloxacin was further diluted with Water to obtain the sub-stock in the range of 10-50 µg/ml. Water was used as a blank solution. The resulting solutions were then scanned in UV spectrophotometer from 200 to 400nm.

Analysis of Oral Dosage Forms

Tablets were crushed to a fine powder a quantity equivalent to 10mg of Enrofloxacin and Ciprofloxacin were transferred to a 10ml volumetric flask separately. Dissolved it in acetone + ethanol 1:1 solution and Water and make up the volume with acetone + ethanol 1:1 solution and Water then sonicated for five minutes and the volume were made up with acetone + ethanol 1:1 solution and Water. The resulting solutions contain 1mg/ml of the drug. The stock solutions of Enrofloxacin and Ciprofloxacin tablets were further diluted with acetone + ethanol 1:1 solution and Water to obtain the sub-stock in the range of 2-10 and 10-50µg/ml. acetone + ethanol 1:1 solution and Water were used as blank solutions. The resulting solutions were then scanned in UV spectrophotometer from 200 to 400nm.

Determination of wavelength of maximum absorption (λmax)

The solution was scanned in the range of 200 to 400 nm to fix the maximum wave length and UV spectrum was obtained.

Validation of the method

Present study was conducted to obtain a new, affordable, cost-effective and convenient method for spectroscopic determination of Enrofloxacin and Ciprofloxacin. The method was developed and validated according to the analytical procedure as per the ICH guidelines for validation of analytical procedures to determine linearity, accuracy, precision, ruggedness, robustness, LOD and LOQ for the analytes i.e. Enrofloxacin and Ciprofloxacin.

Specificity

Specificity of the method was determined by the spectrum of standard Enrofloxacin and Ciprofloxacin.

Linearity

An analytical method's linearity is its ability to produce test results that are proportional to the concentration of the analytes within a given range in samples. The linearity of measurement was evaluated by analyzing different concentration of the standard solution of Enrofloxacin and Ciprofloxacin. Calibration curves were built and the suggested technique was assessed in the respective statistical study by its correlation coefficient and intercept value calculated. For both the method, the Beer Lambert's concentration range was found to be 2-10 and 10-50 µg/ml.

Precision

An analytical procedure's accuracy reflects the proximity of agreement (degree of scattering) between sequences of measurements acquired under the prescribed circumstances from the various sampling of the same homogeneous sample. Precision can be taken into account at three levels: repeatability, intermediate (intraday) precision and reproducibility (interday precision).

- **Intraday Precision:** Solutions containing 06 µg/ml of Enrofloxacin and 30µg/ml Ciprofloxacin were analyzed three times on the same day and %R.S.D was calculated.
- **Interday Precision:** Solutions containing 06 µg/ml of Enrofloxacin and 30µg/ml Ciprofloxacin were analysed on three different successive days and %R.S.D was calculated.
- **Repeatability:** Method precision of the experiment was performed by preparing the standard solution of Enrofloxacin and Ciprofloxacin (06 and 30 µg/ml) for three times and analyzed as per the proposed method.

Ruggedness study

The ruggedness of the method was determined by carrying out the analysis using two different analysts and the respective absorbance was noted. Ruggedness of the methods was assessed by carrying out assay 3 reading with different analyst by using same equipment.

Robustness study

To determine the robustness, the same procedure was carried out by changing the temperature and the result is compared with the same previous procedure.

Limit of Detection (LOD): The detection limit can be calculated using the following equation in accordance with ICH rules.

$$LOD = 3.3 \times (N / S)$$

Where, N = Standard deviation of the drug's peak areas S = Slope of the respective calibration curve.

Limit of Quantification (LOQ): The quantification limit can be calculated using the following equation in accordance with ICH rules. **(kumar *et al.* 2010)**

$$LOQ = 10 \times (N / S)$$

Where, N = Standard deviation of the drug's peak areas S = Slope of the respective calibration curve.

Percentage recovery for the Enrofloxacin and Ciprofloxacin marketed tablet formulation

ICH defines the accuracy of an analytical procedure as the closeness of agreement between the conventional true value or an accepted reference value and the value found. Accuracy may be estimated from the recovery of a known standard solution "spiked" or added into the sample. That is, a known amount of the same substance that is to be tested is added to an aliquot of the sample, usually as a solution, prior to the analysis. The concentration of the analyte in the spiked solution of the sample is then measured. The percent spike recovery is then calculated.

$$\% \text{ Recovery} = \frac{100\ (X_s - X_u)}{K}$$

Where,

Xs= Measured value for the spiked sample

Xu= Measured value for the unspiked sample adjusted for the dilution of the spike K= Known value of spike in the sample.

To check the degree of accuracy of the method, recovery studies were performed in triplicate bystandard addition method at 50%, 100% and 150%. Known amounts of standard were added topre-analyzed samples and were subjected to the proposed method.

RESULTS

Pre-formulation Studies of Enrofloxacin and Ciprofloxacin

Organoleptic Properties

Table: Organoleptic Properties of Enrofloxacin and Ciprofloxacin.

Sr. no.	Parameters	Enrofloxacin	Ciprofloxacin
1	Colour	Pale yellow	Faint to light yellow
2	Odour	Odourless	Odourless
3	Apperance	Solid	solid
4	State	Crystals	Crystals

Solubility study of Enrofloxacin and Ciprofloxacin

Table: Solubility Studies of Enrofloxacin and Ciprofloxacin.

Sr. no.	Solvents	Solubility of Enrofloxacin	Solubility of Ciprofloxacin
1	Methanol	Slightly soluble	Slightly soluble
2	Ethanol	Slightly soluble	Slightly soluble
3	Acetone	Slightly soluble	Slightly soluble
4	Ethanol + Acetone 1:1	Freely soluble	Slightly soluble
5	Water	Sparingly soluble	Freely Soluble
6	Hydrochloric acid (0.1 N)	Slightly soluble	Soluble
7	Acetic acid	Sparingly soluble	Soluble
8	DMSO	Soluble	Sparingly soluble
9	DMF	Soluble	Sparingly soluble

Determination of Melting Point

Table: Melting Point of Enrofloxacin and Ciprofloxacin.

Sr. no.	Drugs	Melting Point Range (as a Reference)	Melting Point
1	Enrofloxacin	219-221 ºC	220 ºC
2	Ciprofloxacin	225-257 ºC	235 ºC

Determination of pH

Table: pH determination.

S. No.	Drug	Observed
1	Enrofloxacin	7.34
2	Ciprofloxacin	3.81

Identification of pure drug

Fourier transform Infrared spectroscopy (FTIR) of Enrofloxacin

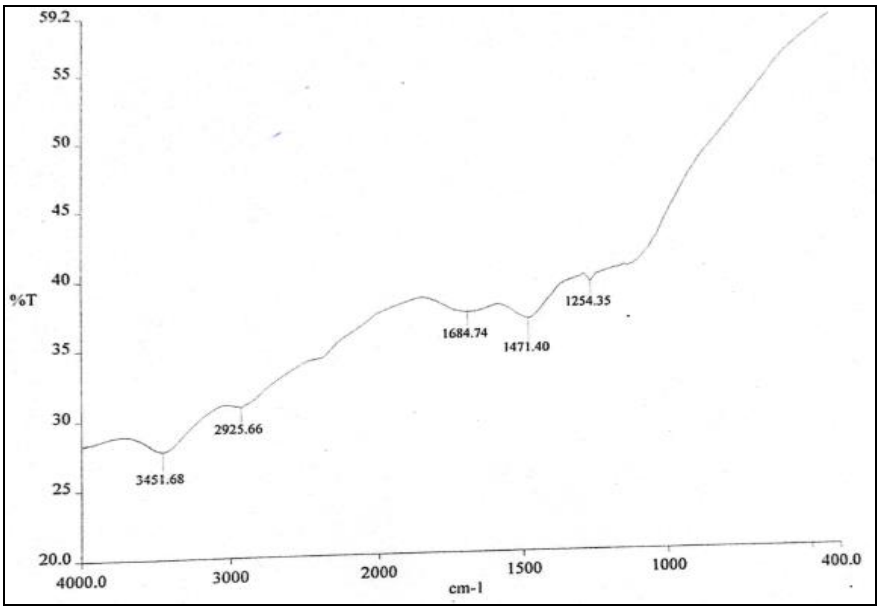

Figure 7: FTIR Spectra of Enrofloxacin.

Table: Interpretation of IR spectrum of Enrofloxacin.

S. No.	Peak obtained	Reference peak	Functional group	Name of functional group
1	3451.68	3500- 3400	O-H stretching	Hydroxyl group
2	2925.66	3000-2840	C-H stretching	alkane
3	1684.74	1690-1640	C=O stretching	Carbonyl group
4	1471.40	1500–1400	C–C stretch (in–ring)	aromatics
5	1254.35	1335–1250	C-N stretching	aromatic amine

FTIR spectra of Enrofloxacin represents Hydroxyl group O-H stretching, broad peak appeared at 3451.68 cm-1, C-H stretching peaks of Alkane at 2925.66. The C=O stretching peak of Carbonyl group at 1684.74 cm-1 C–C stretch peak at 1471.40 cm-1, C-N stretching peak of aromatic amine at 1254.35 cm-1.

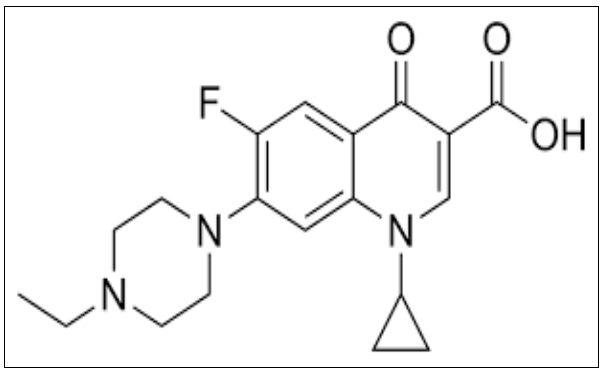

Figure: 8.Enrofloxacin.

Fourier transform Infrared spectroscopy (FTIR) of Ciprofloxacin

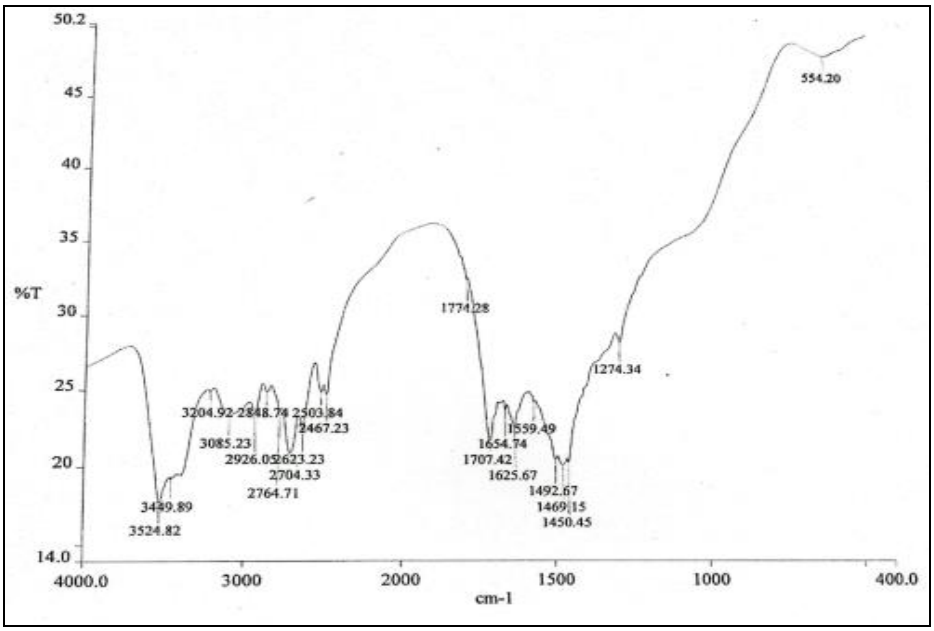

Figure: FTIR Spectra of Ciprofloxacin.

Table: Interpretation of IR spectrum of Ciprofloxacin.

S. No.	Peak obtained	Reference peak	Functional group	Name of functional group
1	3524.82	3550-3200	O-H stretching	Hydroxyl group
2	2926. 05	3000-2840	C-H stretching	Alkane group
3	1707.42	1750-1700	C=O stretching	Carbonyl group
4	1654.74	1703-1623	C=O stretching	Carboxylic acids
5	1559.49	1588–1495	C=C stretching	Aromatic ring
6	1274.34	1300-1250	O-H Bending	Hydroxyl group

FTIR spectra of Ciprofloxacin represent Hydroxyl group O-H stretching, broad peak appeared at 3524.82 cm-1, C-H stretching peaks of Alkane at 2926. 05. The C=O stretching peak of Carbonyl group at 1707.42cm-1, C=O stretching peak Carboxylic acids at 1654.74cm-1, C=C stretching peak of Aromatic ring at 1559.49cm-1, O-H Bending peak of Hydroxyl group at 1274.34cm-1.

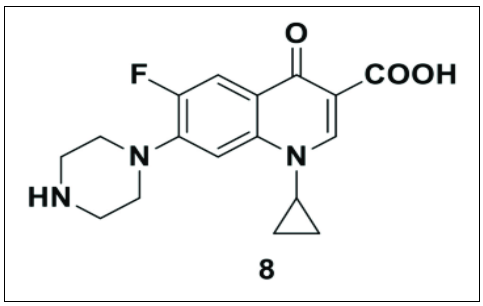

Figure: Ciprofloxacin.

UV-Vis Spectroscopy Method

Determination of absorption maxima

A solution of 6 and 30 µg/mL of Enrofloxacin and Ciprofloxacin and were scanned in the range of 200 to 400 nm. The drugs exhibited the λ max at 276 and 273nm in Ethanol + Acetone 1:1 and Water have good reproducibility graph.

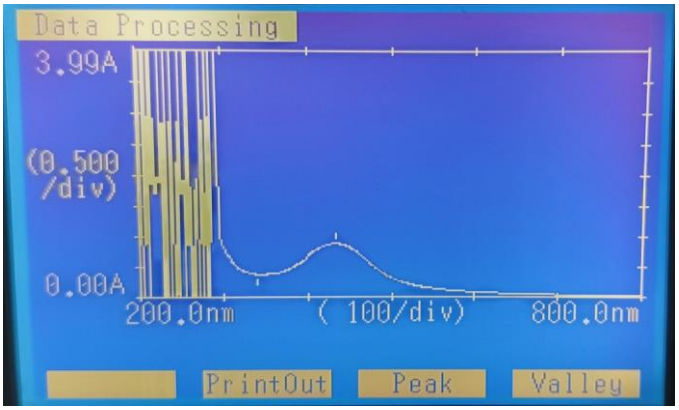

Figure: Absorption maxima of Enrofloxacin (276.0 nm).

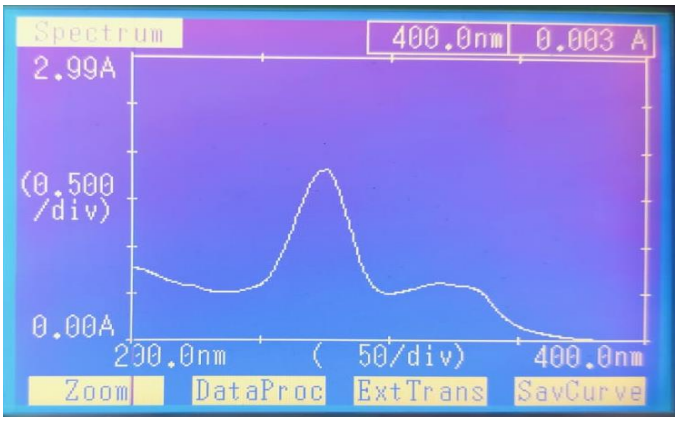

Figure: Absorption maxima of Ciprofloxacin (273.0 nm).

Callibration curve (Linearity)

Calibration Curve (Linearity) of Enrofloxacin

The UV calibration curve of Enrofloxacin was constructed as 1mL of the standard stock solution was taken and diluted to 10mL with Ethanol + Acetone 1:1 solution. Serial dilutions of the stock solutions were prepared and their absorbance values were measured using an ultraviolet–visible (UV- VIS) spectrophotometer at λmax 276 nm. Linearity was observed over a concentration range of 2-10 µg/mL. The absorbance values of different concentration of Enrofloxacin at 276 nm wavelength are given in Table Below.

Table: Calibration data of Enrofloxacin at 276 nm.

Concentration (µg/mL)	Absorbance at 276 nm
2	0.114
4	0.143
6	0.184
8	0.232
10	0.267
Mean	0.268
SD	0.056
%RSD	20.89

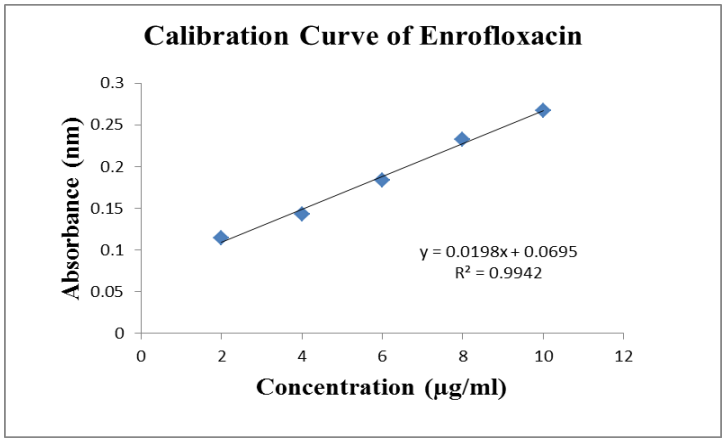

Figure: Calibration curve of Enrofloxacin at 276nm.

The linearity of the proposed method was established by least squares linear regression analysis of the calibration curve. The regression equation for Enrofloxacin was obtained by plotting absorbance versus concentration of the compound in the range of 2-10 µg/ml. The regression equation were y= 0.039x - 0.0408 (Enrofloxacin). The regression coefficient of Enrofloxacin and Ciprofloxacin was R^2 = 0.9973 calculated. Five points calibration curve were obtained in concentration range from 2-10 µg/ml for sample.

Calibration Curve (Linearity) of Ciprofloxacin

The UV calibration curve of Ciprofloxacin was constructed as 1mL of the standard stock solution was taken and diluted to 10mL with dil. water. Serial dilutions of the stock solutions were prepared and their absorbance values were measured using an ultraviolet–visible (UV-VIS) spectrophotometer at λmax 273 nm. Linearity was observed over a concentration range of 10-50 µg/mL. The absorbance values of different concentration of Ciprofloxacin at 273 nm wavelength are given in Table below.

Table: Calibration data of Ciprofloxacin at 273 nm.

Concentration (µg/mL)	Absorbance at 273nm
10	0.248
20	0.459
30	0.648
40	0.846
50	1.001
Mean	0.6404
SD	0.2996
%RSD	46.78

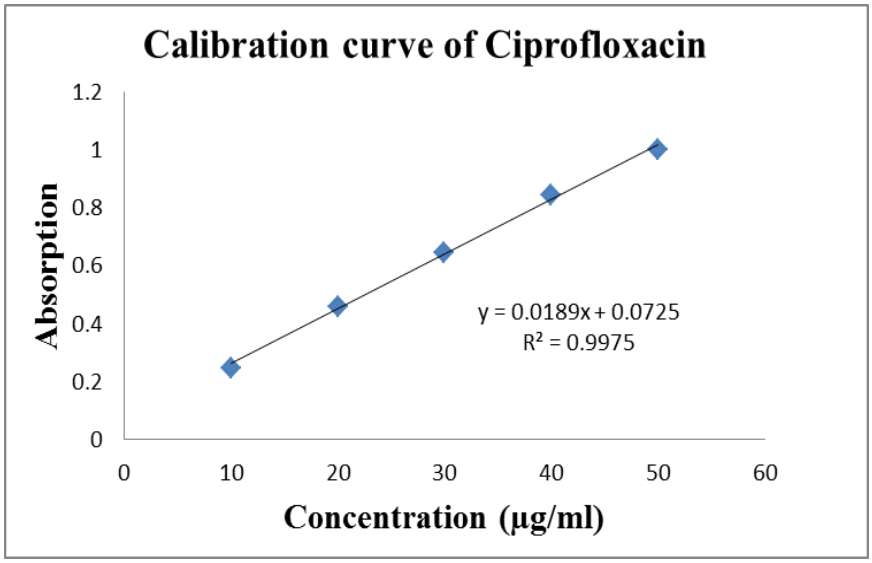

Figure: Calibration curve of Ciprofloxacin at 273 nm.

The linearity of the proposed method was established by least squares linear regression analysis of the calibration curve. The regression equation for Ciprofloxacin was obtained by plotting absorbance versus concentration of the compound in the range of 10-50 µg/ml. The regression equation were y= 0.0189x + 0.0725 (Ciprofloxacin). The regression coefficient of Enrofloxacin and Ciprofloxacin was R2 = 0.9975 calculated. Five points calibration curve were obtained in concentration range from 10-50 µg/ml for sample.

Method Validation via UV spectroscopy for Enrofloxacin and Ciprofloxacin marketed preparation

Precision study

Intraday Precision

Table: Result of Intraday Precision (three times on the same day) of Enrofloxacin.

Concentration (µg/mL)	Day 1 Absorbance (1) at 276 nm	Day 1 Absorbance (2) at 276 nm	Day 1 Absorbance (3) at 276nm
06	0.165	0.167	0.166
06	0.162	0.164	0.165
06	0.163	0.162	0.164
Mean	0.163	0.164	0.165
SD	0.001528	0.002517	0.001
%RSD	0.935	1.531	0.606
AVG % R.S.D	1.024		

Table: Result of Intraday Precision (three times on the same day) of Ciprofloxacin.

Concentration (µg/mL)	Day 1 Absorbance (1) at 273nm	Day 1 Absorbance (2) at 273nm	Day 1 Absorbance (3) at 273 nm
30	0.541	0.545	0.543
30	0.542	0.544	0.545
30	0.543	0.542	0.544
Mean	0.542	0.543	0.544
SD	0.001	0.001528	0.001
%RSD	0.184	0.280	0.183
AVG % R.S.D	0.400		

Interday Precision

Table: Result of Interday Precision (three times on the different day) of Enrofloxacin.

Concentration (µg/mL)	Day 1 Absorbance at 276 nm	Day 2 Absorbance at 276 nm	Day 3 Absorbance at 276nm
06	0.168	0.170	0.167
06	0.165	0.168	0.164
06	0.166	0.164	0.162
Mean	0.166	0.167	0.164
SD	0.001528	0.003055	0.002517
%RSD	0.918	1.825	1.531
AVG % R.S.D	1.424		

Table: Result of Interday Precision (three times on the different day) of Ciprofloxacin.

Concentration (µg/mL)	Day 1 Absorbance at 273 nm	Day 2 Absorbance at 273 nm	Day 3 Absorbance at 273 nm
30	0.548	0.540	0.547
30	0.545	0.548	0.544
30	0.546	0.544	0.542
Mean	0.546	0.544	0.544
SD	0.001528	0.004	0.002517
%RSD	0.279	0.735	0.462
AVG % R.S.D	0.772		

Repeatability

Table: Result of repeatability of Enrofloxaci.

Sr. No.	Concentration (µg/ml)	Absorbance	Statistical analysis	
1	06	0.167	**Mean**	0.164
2	06	0.162	**SD**	0.003011
3	06	0.164	**% RSD**	1.828
4	06	0.161		
5	06	0.169		
6	06	0.165		

Table: Result of repeatability of Ciprofloxacin.

Sr. No.	Concentration (µg/ml)	Absorbance	Statistical analysis	
1	30	0.547	**Mean**	0.544
2	30	0.542	**SD**	0.002517
3	30	0.544	**% RSD**	0.462
4	30	0.541		
5	30	0.549		
6	30	0.545		

Ruggedness

Table: Result of ruggedness of Enrofloxacin.

Analyst-1		Analyst-2	
Concentration (µg/ml)	**Absorbance**	**Concentration (µg/ml)**	**Absorbance**
06	0.168	06	0.172
06	0.167	06	0.175
06	0.170	06	0.173
Mean	0.168	**Mean**	0.173
SD	0.001528	**SD**	0.001528
% RSD	0.907	**% RSD**	0.881

Table: Result of ruggedness of Ciprofloxacin.

Analyst-1		Analyst-2	
Concentration (µg/ml)	**Absorbance**	**Concentration (µg/ml)**	**Absorbance**
30	0.548	30	0.542
30	0.547	30	0.545
30	0.540	30	0.543
Mean	0.545	**Mean**	0.543
SD	0.004359	**SD**	0.001528
% RSD	0.799	**% RSD**	0.281

Robustness

Table: Results showing robustness of Enrofloxacin.

Temperature 25^0C		Temp 30^0C	
Concentration (µg/ml)	**Absorbance**	**Concentration (µg/ml)**	**Absorbance**
06	0.168	06	0.170
06	0.165	06	0.173
06	0.167	06	0.171
Mean	0.166	**Mean**	0.171
SD	0.001528	**SD**	0.001528
% RSD	0.916	**% RSD**	0.891

Table: Results showing robustness of Ciprofloxacin.

Temperature 25^0C		Temp 30^0C	
Concentration (µg/ml)	**Absorbance**	**Concentration (µg/ml)**	**Absorbance**
30	0.548	30	0.550
30	0.545	30	0.553
30	0.547	30	0.551
Mean	0.546	**Mean**	0.551
SD	0.001527	**SD**	0.001527
% RSD	0.279	**% RSD**	0.277

LOD and LOQ

Table: Results showing LOD and LOQ of Enrofloxacin and Ciprofloxacin.

S. No.	Drug name	Wavelength	LOD (µg/ml)	LOQ (µg/ml)
1	Enrofloxacin	276	57.98	175.71
2	Ciprofloxacin	273	54.92	166.44

Percentage recovery of the Enrofloxacin and Ciprofloxacin marketed preparation

A recovery study was carried out to investigate the accuracy by adding standard drug solutions at three concentration levels (50%, 100%, and 150%) to a pre-analyzed sample.

Table: Percentage recovery of the Enrofloxacin.

Excess amt of Enrofloxacin Added (%)	Concentrati on of sample(µg/ ml)	Theoretical concentration of spiked sample(µg/ml)	Concentration of spiked Sample ±SD(µg/ml)(n=3)	Recovery± SD (%)
50	06	05	12.75±0.01 0	98.42±0.064
100	06	06	18.56±0.011	97.56±0.051
150	06	07	24.45±0.024	99.45±0.58

Table: Percentage recovery of the Ciprofloxacin.

Excess amt of Ciprofloxacin Added (%)	Concentrati on of sample(µg/ ml)	Theoretical concentration of spiked sample(µg/ml)	Concentration of spiked Sample ±SD(µg/ml)(n=3)	Recovery± SD (%)
50	30	25	10.75±0.01 0	98.62±0.061
100	30	30	16.56±0.010	97.76±0.050
150	30	35	22.45±0.022	99.85±0.54

Table: Optical Characteristics and Validation Study of Formulation.

Parameters	Enrofloxacin	Ciprofloxacin
Wavelength λ max nm	276	273
Beer's law limit µg/ml	02-10	10-50
Correlation coefficient (R2)	0.995	0.997
Slope	0.007	0.018
Intercept	0.041	0.072
SD	0.123	0.299
% RSD	64.31	46.78
Precision Intraday (% RSD) Interday (% RSD) Repeatability (% RSD)	1.024 1.424 1.828	0.400 0.772 0.462
Ruggedness Analyst 1 (% RSD) Analyst 2 (% RSD)	0.907 0.881	0.799 0.281
Robustness Temp.25^{0}C (% RSD) Temp.30^{0}C (% RSD)	0.916 0.891	0.279 0.277
LOD (µg/ml)	57.98	54.92
LOQ (µg/ml)	175.71	166.44

Table: Tablet Analysis Data for Enrofloxacin and Ciprofloxacin.

Formulation	Drugs	Label	% Recovery
Tablet	Enrofloxacin	150 mg	98-99.8
Tablet	Ciprofloxacin	500 mg	98-99.9

CONCLUSION

The preformulation study of Enrofloxacin and Ciprofloxacin revealed that both compounds exhibit characteristic organoleptic properties, being pale yellow to faint yellow, odorless, and crystalline solids. Solubility analysis showed Enrofloxacin as sparingly soluble in water,

slightly soluble in ethanol, methanol, and acetone, soluble in DMSO and DMF, and freely soluble in a 1:1 ethanol-acetone mixture. Ciprofloxacin was freely soluble in water, slightly soluble in ethanol, methanol, and acetone, and soluble in acetic acid and 0.1 N hydrochloric acid. The melting points were 220°C and 235°C for Enrofloxacin and Ciprofloxacin, respectively. The pH values of 7.34 and 3.81 for Enrofloxacin and Ciprofloxacin fell within acceptable ranges.

FTIR analysis confirmed the presence of functional groups, including primary amines, alkanes, imines, aromatic amines, aldehydes, and ethers in both compounds. Linearity was established for Enrofloxacin (2-10 µg/mL) and Ciprofloxacin (10-50 µg/mL), with regression equations $y = 0.039x - 0.0408$ ($R^2 = 0.9973$) and $y = 0.0189x + 0.0725$ ($R^2 = 0.9975$), respectively. Precision and ruggedness evaluations indicated %RSD values within acceptable limits across intra-day, inter-day, and analyst variations. Robustness assessments at different temperatures demonstrated consistent results, confirming the method's reliability.

The method's sensitivity was reflected in the LOD (57.98 µg/mL for Enrofloxacin and 54.92 µg/mL for Ciprofloxacin) and LOQ values, alongside high % recovery (98-99.9%) for both drugs. This validated technique is simple, accurate, precise, and robust, making it suitable for routine quality control in pharmaceutical formulations.

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Conflict of Interest

The authors declare no conflict of interest.

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