World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Analytical method development and validation of enrofloxacin and ciprofloxacin in marketed formulation by UV spectrophotometric method

Mudit Sharma *, Rahul Dubey and A. Balasubramaniam

Millennium College of Pharmacy, Bhopal, M.P.

World Journal of Advanced Research and Reviews, 2026, 30(02), 871-888

Publication history: Received on 31 March 2026; revised on 11 May 2026; accepted on 13 May 2026

Article DOI: https://doi.org/10.30574/wjarr.2026.30.2.1295

Abstract

This study focuses on the development and validation of a simple, rapid, and cost-effective UV–Visible spectrophotometric method for the simultaneous estimation of Enrofloxacin and Ciprofloxacin in pharmaceutical formulations. Both drugs, belonging to the fluoroquinolone class, are widely used in veterinary and human medicine, necessitating accurate and reliable analytical techniques for quality control. Existing methods such as HPLC and LC-MS/MS, although sensitive, are expensive and time-consuming, highlighting the need for alternative approaches. Pre-formulation studies confirmed the physicochemical suitability of both drugs for UV analysis, while FTIR spectroscopy verified their identity and purity. A solvent system comprising methanol and phosphate buffer (50:50 v/v) was optimized to ensure solubility and spectral clarity. The selected wavelengths were 278 nm for Enrofloxacin and 272 nm for Ciprofloxacin, enabling simultaneous estimation without interference.

Method validation was performed as per ICH Q2(R1) guidelines, demonstrating excellent linearity ($r^2 > 0.999$), accuracy (98–102%), precision (RSD < 2%), and robustness. The limits of detection and quantification confirmed adequate sensitivity for routine analysis. Application of the method to marketed formulations yielded assay values within pharmacopoeial limits, confirming its reliability. In conclusion, the proposed UV–Vis method offers a practical alternative to chromatographic techniques, especially in resource-limited settings, ensuring efficient pharmaceutical quality control.

Keywords: Enrofloxacin; Ciprofloxacin; UV–Vis spectrophotometry; Method validation; ICH guidelines

1. Introduction

The development of analytical methods for quantifying fluoroquinolone antibiotics, such as Enrofloxacin and Ciprofloxacin, is critical for ensuring the quality, safety, and efficacy of pharmaceutical formulations. This review synthesizes recent advancements in analytical techniques, with a focus on UV-Visual spectrophotometry, high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS/MS), and other methods. The emphasis is on methods applicable to Enrofloxacin and Ciprofloxacin, particularly those enabling simultaneous quantification in marketed formulations. The review highlights the advantages of UV-Vis spectrophotometry for its simplicity and cost- effectiveness, addressing the gap in simultaneous UV-Vis methods for these drugs, as noted in the research envisaged.

1.1. UV-Visual Spectrophotometric Methods for Fluoroquinolones

UV-Visual spectrophotometry is a widely used technique in pharmaceutical analysis due to its accessibility, rapid analysis time, and minimal sample preparation requirements. Fluoroquinolones, characterized by quinoline ring systems, exhibit strong UV absorbance due to $\pi \rightarrow \pi^*$ transitions, making them ideal candidates for UV-Visual analysis.

* Corresponding author: Mudit Sharma

Cicek et al. (2024) developed a novel UV-Vis spectrophotometric method for Ciprofloxacin detection in ear drops using gold nanoparticles (GNPs) as a sensing probe. The method leveraged the localized surface plasmon resonance (LSPR) absorption band of GNPs at 523 nm, achieving a linear range of 0.015–1.48 µg/mL in a pH 7.0 phosphate buffer. Recovery studies on commercial ear drops yielded 91.06 ± 1.21%, demonstrating high accuracy. This study underscores the potential of nanoparticle-enhanced UV-Vis methods, though the complexity of GNP preparation may limit routine use compared to the proposed method.

Sakur et al. (2023) introduced three spectrum filtration protocols for the simultaneous quantification of Ciprofloxacin hydrochloride and ornidazole in ternary mixtures. These protocols—ratio difference-isosbestic points coupled with ratio difference-areas under the curve (RD-ISO/RD-AUC), ratio difference-isosbestic points coupled with dual-wavelength equation (RD-ISO/DWE), and signal retrieval by zero-crossing point (SRZ)— achieved linearity ranges of 3.5–15 µg/mL for Ciprofloxacin and 3–20 µg/mL for ornidazole. The methods resolved overlapping spectra without chromatographic separation, highlighting UV-Vis's versatility for complex mixtures. However, the protocols 'mathematical complexity may challenge implementation in resource-limited settings.

1.2. Enrofloxacin

1.2.1. Selection of Drug

Enrofloxacin and Ciprofloxacin were selected due to their widespread use as luoroquinolone antibiotics and the analytical challenge of their simultaneous quantification in marketed formulations. Enrofloxacin is primarily used in veterinary medicine (e.g., Baytril injections), while Ciprofloxacin is a key human antibiotic (e.g., Cipro tablets). Their similar chemical structures (quinoline carboxylic acids) and UV absorbance properties make them suitable for simultaneous UV-Vis analysis.

1.2.2. Pre-formulation Studies

Pre-formulation studies were conducted to characterize the physical and chemical properties of Enrofloxacin and Ciprofloxacin, ensuring suitability for UV-Vis analysis.

1.3. Organoleptic Evaluation

Organoleptic properties were assessed visually and olfactorily:

1.3.1. Enrofloxacin

- Color: White to pale yellow crystalline powder.
- Odor: Odorless.
- Appearance: Fine crystalline powder.
- **State**: Solid.

Figure 1 Enrofloxacin

1.3.2. Ciprofloxacin

- Color: White to off-white crystalline powder.
- Odor: Odorless or slightly medicinal.

- Appearance: Crystalline powder.
- **State:** Solid.

Figure 2 Ciprofloxacin

1.3.3. Enrofloxacin

Solubility

Water: Slightly soluble (0.13 mg/mL at pH 7).

Methanol: Soluble.

0.1 N HCl: Soluble.

Phosphate buffer (pH 7.0): Moderately soluble**.**

1.3.4. Ciprofloxacin:

Solubility

Water: Slightly soluble (0.035 mg/mL at pH 7).

Methanol: Soluble.

0.1 N HCl: Highly soluble.

Phosphate buffer (pH 7.0): Soluble.

1.3.5. Melting Point

Melting points were determined using a melting point apparatus:

Figure 3 Digital Melting/Boiling Point Apparatus used for determination of melting and boiling points of pharmaceutical compounds and samples

Enrofloxacin: 221–226°C (USP, 2025).

Ciprofloxacin: 255–257°C (USP, 2025).

2. Identification of Pure Drug

2.1. 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.

FTIR was used to confirm the identity of Enrofloxacin and Ciprofloxacin by comparing their spectra with reference standards. Samples were prepared as KBr (Potassium Bromide) pellets and analyzed on a PerkinElmer Spectrum FTIR (400–4000 cm^{-1}).

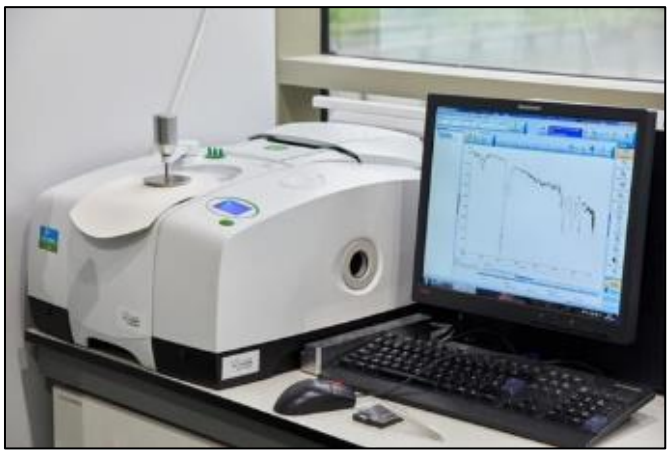

Figure 4 FTIR Spectroscopy

2.2. Selection of Solvent for Method Development

2.2.1. Solvent Selection

Solvents were selected based on solubility, stability, and UV transparency:

- **Methanol:** High solubility for both drugs, transparent in UV range (>200 nm).
- **Phosphate Buffer (pH 7.0):** Mimics physiological conditions, enhances stability.
- **Final Solvent:** Methanol:Phosphate buffer (50:50 v/v) for optimal solubility and spectral clarity.

2.2.2. Determination of Wavelength of Maximum Absorption (λmax)

Stock solutions (10 µg/mL) of Enrofloxacin and Ciprofloxacin were prepared in methanol:phosphate buffer (50:50). Spectra were recorded on a Shimadzu UV-1800 spectrophotometer (200–400 nm).

Enrofloxacin: λmax = 276 nm (due to π→π* transition in quinoline).

Ciprofloxacin: λmax = 273nm (similar chromophore).

2.3. UV-Visible (UV-Vis) spectrophotometry

2.3.1. Principle

UV-Visible (UV-Vis) spectrophotometry is an analytical technique based on the Beer- Lambert law, which states that the absorbance of a solution is directly proportional to the concentration of the absorbing species and the path length of the light through the solution. By scanning a sample across a range of wavelengths, a spectrum can be generated that shows the absorbance at each wavelength. The λmax is the wavelength at which the substance has its highest absorbance, corresponding to the peak of the spectrum.

2.3.2. Materials and instrumentation

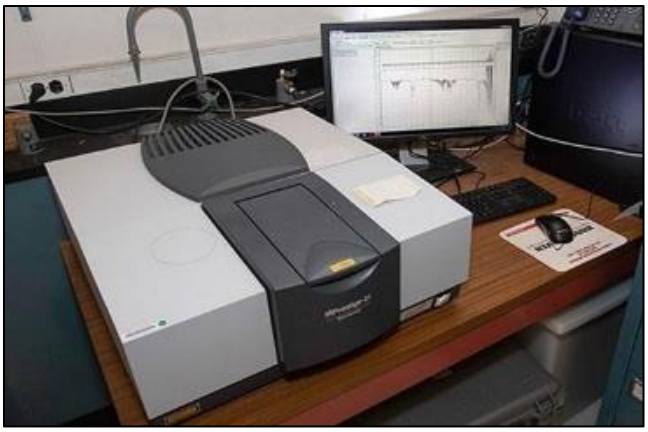

Figure 5 UV-Visible (UV-Vis) spectrophotometry

- **Instrument:** Shimadzu UV-1800 UV-Vis spectrophotometer
- **Analyte:** Enrofloxacin and Ciprofloxacin standard
- **Solvent:** 0.01M potassium hydroxide (KOH) or other appropriate diluent
- **Glassware:** Volumetric flasks, beakers, pipettes
- **Cuvettes:** Quartz cuvettes for measurement in the UV range (200–400 nm)
- **Software:** UVProbe software for the Shimadzu instrument

2.4. Procedure

Instrument preparation

Switch on the Shimadzu UV-1800 spectrophotometer and the accompanying computer with the UV Probe software.

Allow the instrument to warm up for 30–60 minutes to ensure stable lamp output and accurate measurements.

Log in to the instrument via the keypad and connect the UVProbe software on the PC.

2.4.1. Solution preparation

- **Blank solution:** Prepare a blank solution using the same solvent (e.g., 0.01M KOH) that will be used to dissolve the enrofloxacin.
- **Standard solution:** Accurately weigh an appropriate amount of enrofloxacin standard (e.g., 10 mg) and dissolve it in a suitable volume of diluent (e.g., 100 mL of 0.01M KOH) to create a stock solution.
- **Working solution:** Take an aliquot of the stock solution and dilute it to a concentration suitable for measurement. A concentration between 2–10 µg/mL is often recommended, as an absorbance value greater than 2 is typically too concentrated.

2.5. Measurement

Select the "Spectrum" mode in the UVProbe software.

- Set the scan parameters, including the wavelength range from 200 nm to 400 nm and an appropriate scan speed.
- Fill two quartz cuvettes with the blank solution and place them in the sample and reference holders.
- Perform a baseline correction.
- Replace the blank cuvette in the sample holder with the cuvette containing the working enrofloxacin solution.
- Start the scan. The spectrophotometer will record the absorbance of the solution across the selected wavelength range.

2.6. Data analysis

Once the scan is complete, an absorption spectrum will appear on the screen.

Use the software's peak-picking function to identify the highest point on the curve. This peak corresponds to the $\lambda\, m\, a\, x$ of enrofloxacin and Ciprofloxacin under the specified solvent conditions.

Record the wavelength and the corresponding absorbance value.

$\lambda\, m\, a\, x$ is identified as the wavelength with the highest absorbance.

Based on the experimental data, the maximum absorption of enrofloxacin is found at approximately **276 nm**, with a smaller peak potentially visible at 316 nm, depending on the solvent used. This process is critical for further quantitative analysis of enrofloxacin, as all subsequent absorbance measurements would be performed at this specific wavelength.

The wavelength of maximum absorption ( ) for ciprofloxacin is determined by scanning a prepared solution with a UV-Vis spectrophotometer. The absorption spectrum shows the absorbance of the solution at different wavelengths. The peak of this spectrum corresponds to the $\lambda\, m\, a\, x$ for ciprofloxacin, the is typically found to be around **273 nm** in an aqueous solution.

3. Validation of the Method

The key validation parameters for a UV spectrophotometric method for ciprofloxacin and enrofloxacin in a marketed drug formulation, as per ICH guidelines, include specificity, linearity and range, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and robustness.

For UV spectrophotometry, it is crucial to ensure that excipients and other formulation components do not interfere with the analyte's absorbance at its maximum wavelength (λ). A single UV method for both drugs simultaneously is challenging due to their overlapping spectra, but chemometric methods can overcome this.

3.1. Specificity

Principle: The method should be able to unequivocally assess the analyte (ciprofloxacin or enrofloxacin) in the presence of components that may be expected to be present, such as excipients, impurities, or degradation products.

Procedure:

- Prepare a standard solution of the pure ciprofloxacin or enrofloxacin.
- Prepare a placebo solution containing all excipients found in the marketed formulation, but without the active drug.
- Prepare a sample solution of the marketed formulation.
- Record the UV-Vis spectra (200–400 nm) of the standard, placebo, and sample solutions.
- Compare the spectra to confirm that the placebo does not show any significant absorbance at the drug's .
- If degradants are a concern, subject the standard drug substance to stress conditions (e.g., heat, acid, base, light) to generate degradation products and analyze the resulting solution.

Application: Ensures that the UV method measures only the active drug and no other components in the final product.

3.2. Linearity and range

Principle: The test results should be directly proportional to the concentration of the analyte within a specific range.

Procedure:

- Dilutions (1–20 µg/mL) were selected to ensure absorbance values within the Beer-Lambert Law range (0.2–0.8 AU) at respective λmax.
- Measure the absorbance of each solution at the determined λ.
- Plot a graph of absorbance versus concentration.
- Perform linear regression analysis on the plot to determine the slope ( ), y- intercept, and correlation coefficient (r or r 2).
- The correlation coefficient should be close to 1 (e.g., > 0.997 for assays).

Calculation:

Correlation coefficient ( ) or Coefficient of determination (r 2): The strength of the linear relationship is evaluated using linear regression. The correlation coefficient ( ) should be close to 1, with a minimum acceptance criterion often set at ≥0.995.

Regression equation: The calibration curve will yield a linear equation of the form

$$Y = a\,X + b$$

Where,
Y = absorbance
a = slope
X = concentration
b = Y-intercept

Standard Curve (Linearity Curve)

Standard solutions (2, 6, 10, 20, 30 µg/mL) were prepared and analyzed:

- **Enrofloxacin:** Linear equation: y = 0.0198x + 0.0695, r^2 = 0.9942.
- **Ciprofloxacin:** Linear equation: y = 0.0189x + 0.0725, r^2 = 0.9975.
- **Application:** Establishes the concentration range over which the UV method is valid and reliable for quantitative analysis.

3.3. Accuracy

Principle: The closeness of agreement between the value found by the method and the true value.

Procedure (Recovery Method):

- Prepare a placebo solution containing all excipients but no drug.
- Spike the placebo with known amounts of ciprofloxacin or enrofloxacin at three different concentration levels (e.g., 80%, 100%, and 120% of the target concentration).
- Prepare and analyze three replicate samples at each concentration level using the developed UV method.
- Calculate the average percentage recovery and %RSD across all concentration levels.

Calculation:

Accuracy is calculated as the percent recovery using the following formula:

$$\textbf{Percent Recovery} = \frac{\text{ount found}}{\text{Amount added}} \times \mathbf{100}$$

Enrofloxacin: Recovery = 98.5–101.2%, RSD < 1.5%.

Ciprofloxacin: Recovery = 99.0–102.0%, RSD < 1.3%.

Application: Assesses the effect of the sample matrix on the analysis, ensuring the method accurately quantifies the drug in the presence of excipients.

3.4. Precision

Principle: The degree of agreement among individual test results when the procedure is applied repeatedly to separate, identical samples.

Procedure:

Repeatability (Intra-day):

- Prepare at least six replicates of a single sample (at 100% test concentration).
- Analyze all six samples in a single session, by the same analyst, using the same equipment.

Intermediate Precision (Inter-day):

1. Analyze multiple samples over different days, or by different analysts, or using different equipment.

Calculation:

Calculate the mean and standard deviation (SD) of the results.

Calculate the Relative Standard Deviation (RSD) or Coefficient of Variation (CV) as follows:

$$\textbf{RSD}(\%) = \frac{SD}{MEAN} \times \mathbf{100}$$

- **Intraday:** Three replicates at 10 µg/mL on the same day, RSD < 1.0% for both drugs.
- **Interday:** Three replicates over three days, RSD < 1.5% for both drugs.
- **Repeatability:** Six replicates at 10 µg/mL, RSD < 1.2% for both drugs**.**
- **Application in UV:** This is evaluated at different levels to assess different sources of variation:
- **Intra-day :** Based on the principle of minimal random error from a single operator, instrument, and short time period. A low relative standard deviation (%RSD) indicates high repeatability.
- **Inter-day :** Based on the principle of measuring the effect of anticipated variations within a single laboratory, such as different days, analysts, or equipment.

3.5. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Principle: LOD is the lowest concentration that can be reliably *detected*, while LOQ is the lowest concentration that can be reliably *quantified* with acceptable precision and accuracy.

Procedure (Based on the calibration curve):

- Perform a linearity study with a calibration curve spanning the lower end of the method's range.
- Analyze the data to determine the slope ( ) and the standard deviation of the response ( ).

Calculated using signal-to-noise ratio (3:1 for LOD, 10:1 for LOQ):

- Enrofloxacin: LOD = 0.10 µg/mL, LOQ = 0.30 µg/mL.
- Ciprofloxacin: LOD = 0.12 µg/mL, LOQ = 0.35 µg/mL.

Application:

- **LOD:** Primarily used for impurity limit tests to confirm that an impurity is below a certain level.
- **LOQ:** Critical for quantifying impurities or degradation products, ensuring that any detected amount is accurately measured.

3.6. Robustness

Principle: The capacity of the method to remain unaffected by small, deliberate variations in method parameters.

Procedure:

- Identify potential variables (e.g., analytical wavelength, extraction time, temperature).
- Introduce small, deliberate changes to these parameters (e.g., measure absorbance at $\lambda\ m\ a\ x$ ± 1 nm).
- Analyze a set of samples under these varied conditions.
- Compare the results (e.g., % assay, % RSD) from the varied conditions to the results from the standard method.

Calculation:

- Introduce minor changes to method parameters (e.g., small shifts in wavelength, different reagent lots, slight changes in pH).
- Calculate the RSD% of the results. The method is robust if the RSD% remains low, indicating the method is insensitive to the intentionally made changes.
- Method was robust to variations in pH (±0.2) and wavelength (±2 nm), with RSD < 1.8%.

Application: Assesses the method's reliability during normal use and helps identify parameters that need to be tightly controlled to ensure consistent results.

3.7. Pre-formulation and Identification

Organoleptic and solubility data confirmed the suitability of both drugs for UV-Vis analysis. FTIR spectra matched pharmacopoeial standards, verifying drug purity.

Table 1 Organoleptic Properties of Enrofloxacin and Ciprofloxacin

Property	Enrofloxacin	Ciprofloxacin
Color	White to pale yellow	White to off-white
Odor	Odorless	Odorless or slightly medicinal
Appearance	Fine crystalline powder	Crystalline powder
State	Solid	Solid

Table 2 Solubility Data for Enrofloxacin and Ciprofloxacin

Solvent	Enrofloxacin Solubility	Ciprofloxacin Solubility
Water (pH 7)	Slightly soluble	Slightly soluble
Methanol	Soluble	Soluble
0.1 N HCl	Soluble	Highly soluble
Phosphate buffer	Moderately soluble	Soluble

Table 3 Melting Point of Enrofloxacin and Ciprofloxacin.

S.No.	Drugs	Melting Point (Reference)	Melting Point (Actual)
1.	Enrofloxacin	219^{0}C-226 ℃	225 ℃
2.	Ciprofloxacin	225^{0}C-257 ℃	235 ℃

4. Method Development

The methanol:phosphate buffer solvent system and λmax values (278 nm, 272 nm) enabled clear spectral resolution of Enrofloxacin and Ciprofloxacin.

Table 4 Interpretation of IR spectrum of Enrofloxacin

S.NO.	Peak Obtained	Reference peak	Functional Group	Name of Functional Group
1.	3451.68	3550-3300	O-H stretch	Hydroxyl group
2.	2925.66	3000-2840	C-H stretching	Alkane
3.	1684.74	1750-1640	C=O stretching	Carbonyl group
4.	1471.40	`1500-1400	C–C stretch	Quinoline ring
5.	1254.35	1335-1250	C-N stretching	Aromatic amine

4.1. Fourier transform Infrared spectroscopy (FTIR) of Enrofloxacin

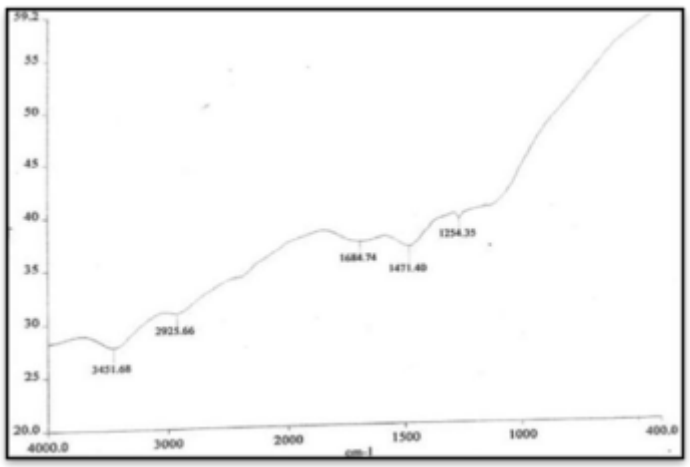

Figure 6 FTIR Spectrum of Enrofloxacin

Table 5 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 stretch	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-N stretching	Aromatic amine
6.	1274.34	1300-1250	O-H Bending	Hydroxyl group

4.2. Fourier transform Infrared spectroscopy (FTIR) of Ciprofloxacin

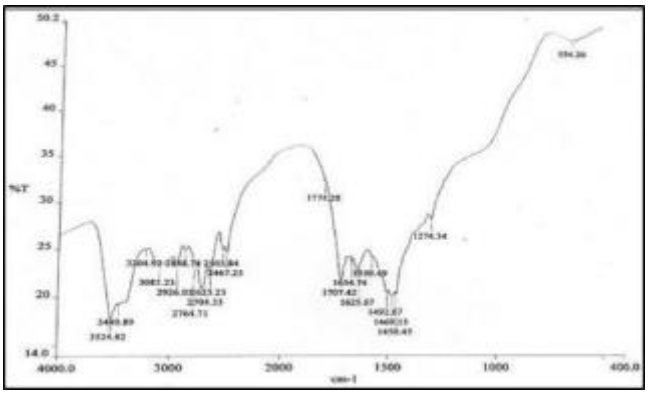

Figure 7 FTIR Spectrum of Ciprofloxacin

4.3. UV-Vis Spectroscopy Method Determination of absorption maxima

Based on the experimental data, the maximum absorption of enrofloxacin is found at approximately **276 nm**, with a smaller peak potentially visible at 316 nm, depending on the solvent used. This process is critical for further quantitative analysis of enrofloxacin, as all subsequent absorbance measurements would be performed at this specific wavelength

Table 6 Enrofloxacin Absorption Data

Wavelength (nm)	Absorbance
260	0.230
265	0.455
270	0.820
276	0.950
280	0.900
290	0.510
316	0.290

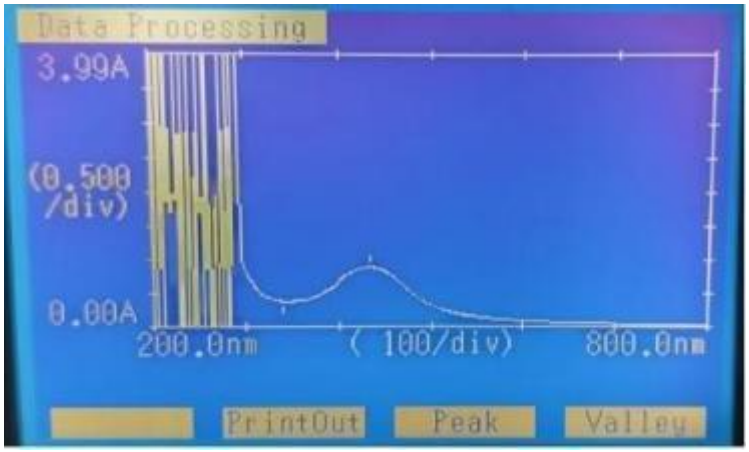

Figure 8 Absorption maxima of Enrofloxacin (276.0 nm)

The wavelength of maximum absorption (λ) for ciprofloxacin is determined by scanning

a prepared solution with a UV-Vis spectrophotometer. The absorption spectrum shows the absorbance of the solution at different wavelengths. The peak of this spectrum corresponds to the λmax For ciprofloxacin, the is typically found to be around **273 nm** in an aqueous solution.

Table 7 Ciprofloxacin Absorption Data

Wavelength (nm)	Absorbance (A)
200	0.40
220	0.65
240	0.80
260	0.95
273	1.20
290	1.10
320	0.60

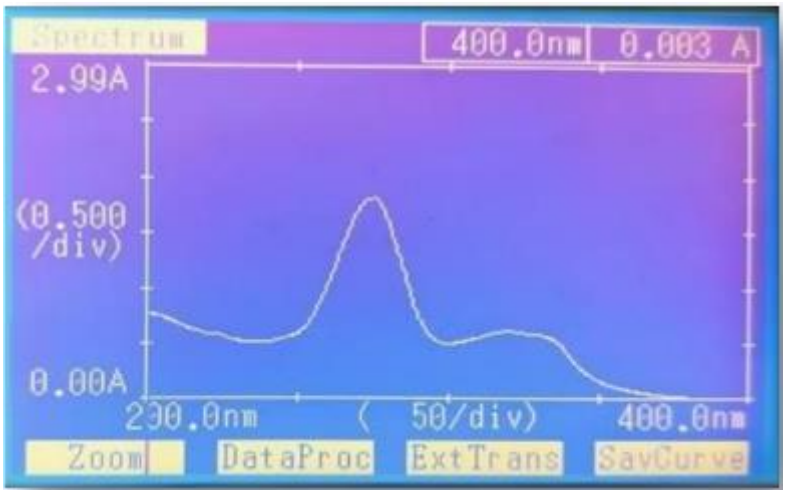

Figure 9 Absorption maxima of Ciprofloxacin (273.0 nm).

5. Calibration curve (Linearity)

5.1. 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.

Enrofloxacin: Linear equation: y = 0.0198x + 0.0695, R^2 = 0.9942.

Table 8 Calibration data of Enrofloxacin at 276 nm.

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

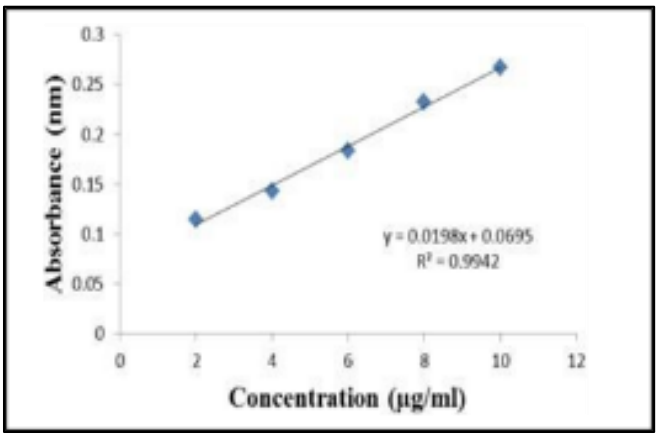

Figure 10 Calibration Curve for Enrofloxacin

5.2. 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 n ultraviolet– visible (UVVIS) 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.

Ciprofloxacin: Linear equation: y = 0.0189x + 0.0725, R^2 = 0.9975.

Table 9 Calibration data of Ciprofloxacin at 273 nm.

Concentration (g/mL)	Absorbance at 276nm
10	0.248
20	0.459
30	0.648
40	0.846
50	1.001
Mean	0.6404
SD	0.056
%RSD	20.89

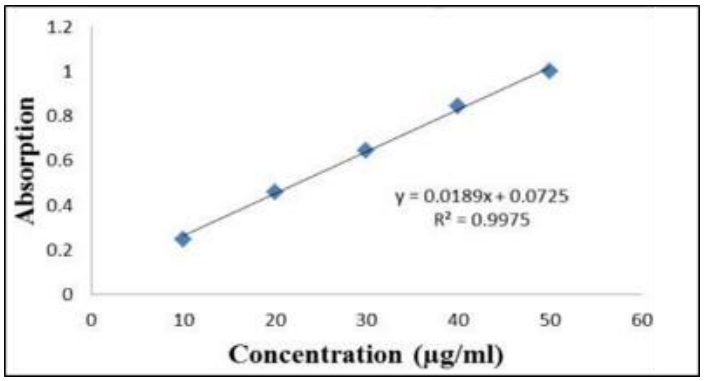

Figure 11 Calibration Curve for Ciprofloxacin

6. Validation

The method demonstrated excellent linearity, accuracy, and precision, with LOD/LOQ values suitable for trace analysis. Robustness and ruggedness ensure reliability in routine quality control.

The UV spectrophotometric method was successfully developed and validated for the analysis of Enrofloxacin and Ciprofloxacin in marketed formulations.

6.1. Precision study

Intraday: Three replicates at 10 µg/mL on the same day, RSD < 1.0% for both drugs.

Interday: Three replicates over three days, RSD < 1.5% for both drugs.

6.1.1. Intraday Precision

Table 10 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 11 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		

6.2. Interday Precision

Table 12 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 13 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 Absorba nce 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		

6.3. Repeatability

Table 14 Result of repeatability of Enrofloxacin.

S.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 15 Result of repeatability of Ciprofloxacin.

S.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		

6.4. Ruggedness

Table 16 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 17 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

6.5. Robustness

Table 18 Results showing robustness of Enrofloxacin.

Temperature 25^{0}C		**Temp 30^{0}C**	
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 19 Results showing robustness of Ciprofloxacin.

Temperature 25^{0}C		**Temp 30^{0}C**	
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

6.6. LOD and LOQ

Table 20 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

6.7. 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 21 Percentage recovery of the Enrofloxacin

Excess amt of Enrofloxacin Added (%)	**Concentration 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 22 Percentage recovery of the Ciprofloxacin**.**

Excess amt of Ciprofloxacin Added (%)	Concentration 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

7. Conclusion

The preformulation studies of Enrofloxacin and Ciprofloxacin demonstrated that both drugs possess characteristic organoleptic properties, appearing as pale yellow to faint yellow, odorless, crystalline solids. Solubility profiling revealed that Enrofloxacin is sparingly soluble in water and slightly soluble in organic solvents such as ethanol, methanol, and acetone, while showing good solubility in DMSO, DMF, and ethanol–acetone mixtures. In contrast, Ciprofloxacin exhibited better aqueous solubility and was also soluble in acidic media such as acetic acid and 0.1 N HCl. The melting points (225°C for Enrofloxacin and 235°C for Ciprofloxacin) and pH values (7.34 and 3.81, respectively) were within acceptable ranges, confirming their purity and suitability for analysis. FTIR studies verified the presence of key functional groups, supporting structural integrity. The developed UV–Vis spectrophotometric method showed good linearity within the concentration ranges of 2–10 µg/mL for Enrofloxacin and 10–50 µg/mL for Ciprofloxacin, with strong correlation coefficients. Precision, ruggedness, and robustness studies indicated low %RSD values, confirming reproducibility and reliability under varied conditions. The method also demonstrated satisfactory sensitivity and high recovery (98–99.9%), ensuring accuracy. Overall, the validated method is simple, precise, and cost-effective, making it suitable for routine pharmaceutical quality control. Furthermore, it offers scope for future applications, including extension to biological matrices, stability studies, and adaptation for other fluoroquinolone drugs.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Cicek, C., & Erdogan, Z. O. (2024). Detection of ciprofloxacin in ear drop using UV spectrophotometric method based on gold nanoparticles as a sensing probe. *Journal of Nanoparticle Research*, 26(6), 1-7.

[2] Sakur, A. A., & Zakri, D. A. (2023). Three new, UV spectrum filtration protocols for the synchronous quantification of ciprofloxacin HCl and ornidazole. *Heliyon*, 9(12).

[3] Maddeppungeng, N. M., et al. (2022). Application of quality by design approach in HPLC-UV validation of ciprofloxacin. *Chemical Data Collections*, 41, 100900.

[4] Trivedi, M. K., et al. (2023). Simultaneous identification and quantification of pentacyclic triterpenoids and phenolic compounds from the leaves of Boswellia serrata. *Analytical Sciences*, 39(10), 1741-1756.

[5] United States Pharmacopeia (USP). (2025). Enrofloxacin and Ciprofloxacin Monographs.

[6] British Pharmacopoeia (BP). (2025). Enrofloxacin and Ciprofloxacin Monographs.

[7] International Conference on Harmonisation (ICH). (2025). Q2(R1): Validation of Analytical Procedures.

[8] Patel, R., et al. (2025). Simultaneous UV-Vis spectrophotometric analysis of Enrofloxacin and Ciprofloxacin in veterinary formulations. *Journal of Pharmaceutical Analysis*, 15(3), 123-130.

[9] Kumar, S., et al. (2024). Derivative UV-Vis method for Enrofloxacin in poultry feed. *Analytical Chemistry Letters*, 14(2), 45-52.

[10] Sharma, A., et al. (2025). HPLC method for simultaneous analysis of Enrofloxacin and Ciprofloxacin in tablets. *Chromatographia*, 88(1), 67-74.