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Research Article

Development and Validation of a Simple UV Spectroscopic Method for the Estimation of Enrofloxacin

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ABSTRACT

The present work focused on the development and validation of a simple and cost effective method for estimation of enrofloxacin by UV spectroscopy. Enrofloxacin is an antibiotic used commercially for veterinary purposes. The UV-1700, Shimadzu, Japan was used for the measurements. Methanol and water in the ratio of 7:3 was used as the solvent. Enrofloxacin exhibited absorption maxima at 277 nm in methanol water (7:3). The method was found to be specific since no interference with the blank was observed. The linearity range was found to be 2.01 mcg/ml to 12.12 mcg/ml with regression coefficient of 0.999. The % recovery within the range of 100.34 to 100.62 and the standard deviation and % relative standard deviations are less than 2. The method was found to be precise according to the repeatability data, intraday precision data and interday precision data with the standard deviation and % relative standard deviation less than 2. The method was rugged and robust with the standard deviation and % relative standard deviation less than 2. The proposed method was found suitable, rapid, cost effective, accurate, precise, robust and rugged for assay and routine analysis of enrofloxacin in bulk and its marketed dosage form.

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INTRODUCTION [1-6]

Enrofloxacin is a veterinary antibiotic act by inhibition of deoxyribonucleic acid (DNA) gyrase, thus inhibiting both DNA and ribonucleic acid (RNA) synthesis and it is used to cure urinary tract, respiratory tract and skin infectious diseases in pet or animals. Chemically Enrofloxacin is 1-cyclopropyl-7-(4-ethylpiperazin-1-yl)-6-fluoro-4-oxo-1, 4-dihydroquinolone-3-carboxilic acid.

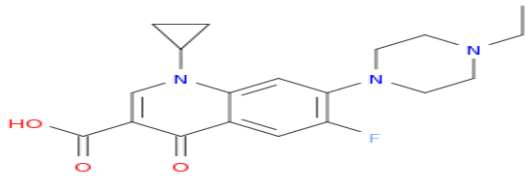

Figure 1: Structure of Enrofloxacin

Dissolution method for enrofloxacin immediate release tablets by UV spectroscopy was developed and validated as per ICH guidelines. Potassium di hydrogen phosphate pH 4.5 was used as solvent. The maximum absorbance was observed at 276nm [7]. Stability-indicating reversed-phase (RP) HPLC method has been developed and validated for quantitative analysis of bromhexine HCl and enrofloxacin in the bulk drug and its marketed oral preparation.[8] Stability-indicating HPLC method was reported for simultaneous determination of amoxicillin and enrofloxacin combination in an injectable suspension.[9] Reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and an isocratic reversed phase high performance liquid chromatographic method with DAD detection was developed for the analysis of oxytetracycline and enrofloxacin.[10] A study was to determine the enrofloxacin residues in broiler's meat and liver samples. Detection of enrofloxacin residues in meat (n = 75) and liver (n = 75) samples was performed by high performance liquid chromatography with UV detector set at 268 nm using C18 column.[11] Stability-indicating reverse-phase high performance liquid chromatographic (RP-HPLC) method was developed for simultaneous determination enrofloxacin and its degradation products including ethylenediamine impurity, desfluoro impurity, ciprofloxacin impurity, chloro impurity, fluoroquinolonic acid impurity, and decarboxylated impurity in tablet dosage forms.[12] UV-spectrophotometric methods have been developed and validated for the assay of enrofloxacin in bulk drug and in its dosage forms by using 0.1N HCl,0.1N NaOH and 0.1M glacial acetic acid.[13] Stability-indicating assay method was developed for the simultaneous determination of enrofloxacin and piroxicam in combination

and in the presence of degradation products by reverse phase high-performance liquid chromatography.[17] An isocratic reverse phase liquid chromatography (RP-LC) method has been developed and subsequently validated for the determination of enrofloxacin and its related substances in bulk and its pharmaceutical tablet formulation.[14] The study describes a simple salting-out liquid–liquid extraction technique for simultaneously detection of ciprofloxacin and enrofloxacin residues in water samples.[16] In literature review a very few works was carried by UV spectroscopy but not used by polar solvents so an attempt was made to develop and validate a simple cost effective method for estimation of enrofloxacin by UV spectroscopy.

EXPERIMENTAL WORK

Optimization

Solubility study: Solubility studies was performed by using various solvents such as distilled water, methanol, 0.1N HCl, 0.1N NaOH and DMSO etc.

Method:

Preparation of Stock Solutions: Standard stock solutions were prepared by dissolving 10 mg of each drug separately in 10 mL of methanol: water (7:3) to get concentration of 1mg/mL (1000 µg/mL) solutions.

Calibration Curve: The prepared stock solutions were further diluted with methanol: water (7:3) to get working standard solutions of 100 µg/mL of the selected drugs. To construct Beer's law plot for pure drug, different aliquots of drug was taken and diluted to 10 mL with methanol: water (7:3). Within the range of 2-12 µg/mL. The calibration curves were plotted by taking concentration of drug on x-axis and absorbance on y-axis with its detection wavelength-277nm.

Estimation of Drug in their Dosage Forms: Transfer equivalent to 8 mg of enrofloxacin powder into a 100 mL volumetric flask, add 50 mL of diluents and sonicated for 15 min with intermediate shaking. Finally volume makeup with diluents. Filter through the 0.45µm membrane filter. Further dilute 10 mL of this solution to 100 mL with diluent and mix. Each sample solution was recorded at respective λ_{max} and concentrations of drugs in the samples were calculated.

Validation

Specificity:

The standard and sample solutions were prepared as per the methodology and scanned by UV spectrophotometer at 277 nm using assay diluents as blank.

Precision

System precision

The standard and sample solutions were prepared as per the method and checked the absorbance for six times into UV Spectrophotometer. The mean, SD and % RSD for absorbance of enrofloxacin were calculated.

Method precision

Assay was performed on six preparations and analyzed as per the test method.

Intermediate precision (Ruggedness)

Assay was performed on six sample preparation and analyzed by different analyst, by using different system on different day.

Calculations were carried out for the % RSD for % Assay of six preparations as per protocol. The overall % RSD for % Assay of above experiment results along with method precision results were calculated.

Stability in analytical solution

Standards were prepared and assay was performed for sample solutions as per test method at Initial and 24 hours on single sample preparation.

Linearity & Range

The linearity of enrofloxacin was determined in the range of 2.01 µg/mL to 12.04µg/mL. A graph was plotted with concentration (in mcg/mL) on X axis and Absorbance of enrofloxacin on Y axis. Slope, y-intercept, and correlation coefficient (r-value) were determined.

Accuracy as recovery

Known amount of enrofloxacin spiked in diluents at about 80 %, 100 %, and 120 % of test concentration. The amount of Enrofloxacin was quantified as per the test method. The % recovery was calculated from the amount found and actual amount added.

Robustness

The assay was performed as per following parameters,

a. By changing the solvents composition (± 10 %)

b. By changing the wavelength 277 ± 2nm

Detection Limit and Quantification Limit:

Calibration curve was plotted by using concentration in the expected detection limit range (0.1-5 µg/mL) for the drug. The standard deviation of y-intercept of regression line were determined and substituted in the following equation for the determination of detection limit and quantification limits.

Detection limit = 3.3 σ/s and

Quantification limit = 10 σ/s

Where σ is the standard deviation of y-intercept of regression line and s is the slope of the calibration curve.

RESULTS AND DISCUSSION

Table 1: Solubility study data of Enrofloxacin

Solvent	Solubility
Dist. Water	Slightly soluble
Ethanol	Soluble
Methanol	Soluble
0.1(N) NaOH	Soluble
0.1N HCl	Soluble

Table 2: Specificity data of Enrofloxacin

Sample	Absorbance	%
Blank	0.000	---
Standard	0.97	NA
Test 400	0.91	NA

Table 3: Method precision data of Enrofloxacin

Sr. No	Absorbance	% Assay of Enrofloxacin
1	0.919	95.22
2	0.918	94.60
3	0.923	94.60
4	0.945	97.38
5	0.935	95.83
6	0.931	95.78
Mean		**95.57**
SD		**1.038**
%RSD		**1.09**

Table 4: Intermediate precision data of Enrofloxacin

% Assay of Enrofloxacin			
Sr.	**Absorbanc**	**Intermedia**	**Method**
1	0.890	96.18	95.22
2	0.880	94.90	94.60
3	0.881	94.55	94.60
4	0.883	95.22	97.38
5	0.856	91.86	95.83
6	0.896	96.73	95.78
Mean		**94.91**	**95.57**
SD		**1.700**	**1.038**
%RSD		**1.79**	**1.09**
Over all Mean		**95.24**	
Over all SD		**1.39**	
Over all %RSD		**1.46**	
Analyst		**Analyst II**	**Analyst**
UV-Vis		**UV-1800**	**UV-**

Table 5: Standard solution stability data of Enrofloxacin

Time (In hours)	Enrofloxacin	
	Absorbance	**%**
Initial	0.971	-
24	0.989	1.9

Table 6: Test solution stability data of Enrofloxacin

Time (In hours)	Enrofloxacin	
	%Assay	**%**
Initial	94.4	-
24 hour	96.2	**1.83**

Table 7: Linearity & Range data of Enrofloxacin

Sr. No	Concentration (µg/mL)	Absorbance
1	2.01	0.229
2	4.01	0.49
3	6.02	0.72
4	8.02	0.97
5	10.03	1.21
6	12.04	1.49
Slope		0.124
Intercept		-0.020
Correlation		0.999

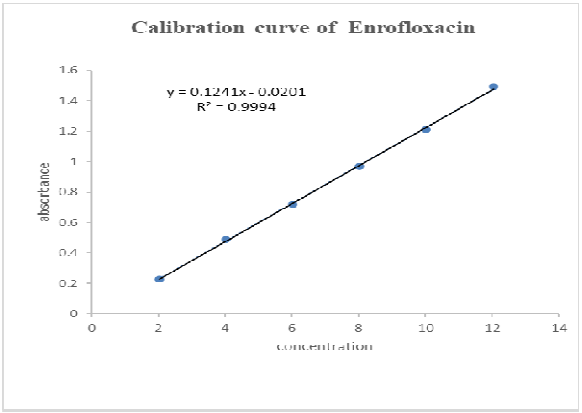

Figure 2: Calibration plot of Enrofloxacin

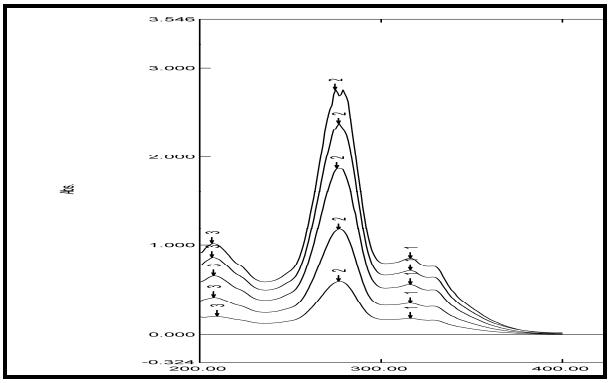

Figure 3: UV-Vis Spectra of Enrofloxacin

Table 8: Optical Characteristics of Enrofloxacin

Beer's Law limit (µg/ml)	2.01-12.12.04µg/ml
Correlation coefficient	0.999
Regression equation (Y)	Y=0.124x-
Slope (a)	0.124
Intercept (b)	-0.020

Table 9: Accuracy as recovery data of Enrofloxacin

Level	Amount Added in(mg)	Abs	Amount Recovered (mg)	% Recovery	Mean % Recovery	SD	% RSD
Level 1 (80%)	6.40	0.785	6.46	100.94	100.62	0.32	0.31
	6.41	0.781	6.43	100.31			
	6.42	0.785	6.46	100.62			
Level 2 (100%)	8.010	0.981	8.07	100.75	100.34	0.72	0.71
	8.100	0.980	8.06	99.51			
	8.030	0.983	8.09	100.75			
Level 3 (120%)	9.60	1.18	9.71	101.15	100.42	0.32	0.31
	9.62	1.19	9.79	101.77			
	9.65	1.189	9.78	101.35			
Over all Mean						**100.79**	
Over all SD						**0.64**	
Over all % RSD						**0.64**	

Table 10: Robustness data of Enrofloxacin data

Parameter	Over all %Assay Standard	% RSD	Over All% RSD Of Assay
Plus	95.37	0.34	0.93
Minus	95.45	0.45	0.91
Plus	95.26	0.50	1.03
Minus	95.07	0.59	1.21

Limit of Detection (LOD):

The LOD for enrofloxacin was found to be 0.207µg/mL.

Limit of Quantitation (LOQ):

The LOQ for enrofloxacin was found to be 1.626µg/mL

CONCLUSIONS

The present work aimed at developing a simple validated quantification method of enrofloxacin by UV spectroscopy. It exhibited absorption maxima at 277 nm in methanol water (7:3). The method was specific as there was no interference with the blank. The linearity range was found 2.01µg/mL to 12.04 µg/mL with regression coefficient of 0.999. The % recovery was within the range of 100.34 to 100.62 and the standard deviation and % relative standard deviations were less than 2. The method was found to be precise according to the repeatability data, intraday precision data and interday precision data with the standard deviation and % relative standard deviation less than 2. The method was rugged and robust with the standard deviation and % relative standard deviation less than 2. The proposed method was found suitable, rapid, cost effective, accurate, precise, robust and rugged for assay routine analysis of enrofloxacin in its marketed dosage form.

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