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RESEARCH ARTICLE

DISSOLUTION METHOD DEVELOPMENT AND VALIDATION OF ENROFLOXACIN TABLETS BY UV SPECTROPHOTOMETRY

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ABSTRACT

The main aim of the article is to develop a simple dissolution method for Enrofloxacin immediate release tablets by UV spectroscopy and validate as per ICH guidelines. The optimized dissolution method includes potassium di hydrogen phosphate pH 4.5 as dissolution media, apparatus as USP Type 2 Paddle, rpm as 100, temperature of dissolution media as 37±0.5ºC, dissolution volume as 500ml, dissolution time point as 30 minutes, working concentration of standard and sample as 5µg/ml and a detection wavelength of 276 nm. The developed method resulted in Enrofloxacin exhibiting linearity in the range 1.25-10µg/ml. System precision and intra-day precision are exemplified by relative standard deviation of 0.148% and 0.950% respectively. Method was found to be rugged/inter day precise as % RSD was found to be 0.924%. Percentage Mean recovery was found to be in the range of 90-110 % by absolute method during accuracy studies. Hence it can be concluded that effective dissolution method by UV spectroscopy is developed and validated which can be applicable in various pharmaceutical industries.

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INTRODUCTION

The rate and extent at which the amount of drug substance is dissolved over a period of time is called dissolution. It is expressed as percentage release of drug substances present in dosage forms such as tablets, capsules, oral suspensions, transdermal patches, suppositories, semi-solid topical preparations and ointments. It describes about manufacturing reproducibility, product performance similarity and biological availability of drug from its formulation. Therefore, it is considered as one of the most quality control test of solid pharmaceutical dosage forms (Foresti *et al.,* 2015).

Enrofloxacin (Figure 1) is a 1-cyclopropyl-7-(4-ethylpiperazin-1-yl)-6-fluoro-4-oxoquinoline-3-carboxylic acid. It has a molecular formula of $C_{19}H_{22}FN_3O_3$ and molecular mass of 359.401 g/mol. Enrofloxacin is a synthetic antibacterial agent from the class of the fluoroquinolone carboxylic acid derivatives. It has antibacterial activity against a broad spectrum of Gram-negative and Gram-positive bacteria. Its mechanism of action is not thoroughly understood, but it is believed to act by inhibiting bacterial DNA gyrase (a type-II topoisomerase), thereby preventing DNA supercoiling and DNA synthesis. Few analytical methods have been reported for the determination of ENROFLOXACIN in various matrices by LCMS (Laa *et al.,* 2011; Ben Salem *et al.,* 2015; Andreia Freitas *et al.,* 2013), UV (Mostafa *et al.,* 2002) and RP-HPLC (Brahmareddy *et al.,* 2015; PavaniPeddi *et al.,* 2016; Jakubowski *et al.,* 2010; Baisakhi Moharana *et al.,* ?; NidalBatrawi *et al.,* 2017; Ashok Chakravarthy *et al.,* 2015; Stability-Indicating, 2015; Prasad *et al.,* 2010). UV dissolution methods were reported where 0.1 N HCl and phosphate buffer of Ph6.8 are used as dissolution media. Our present study aims at developing first dissolution method for Enrofloxacin immediate release tablets by UV spectrophotometry and validate as per ICH guidelines.

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MATERIALS AND METHODS

Instrument

A double beam UV-visible spectrophotometer (Shimadzu, model UV-1800) having two matched quartz cells with 1 cm light path and loaded with UV probe software (version 2.41) was used for recording of spectra and measuring absorbance. Dissolution studies were performed on USP Dissolution apparatus (Electrolab, Model: TDT-08L). An electronic

analytical weighing balance (0.1 mg sensitivity, Shimadzu AY 220), digital pH meter (DELUX model 101), a sonicator (sonica, model 2200 MH) were used in the study.

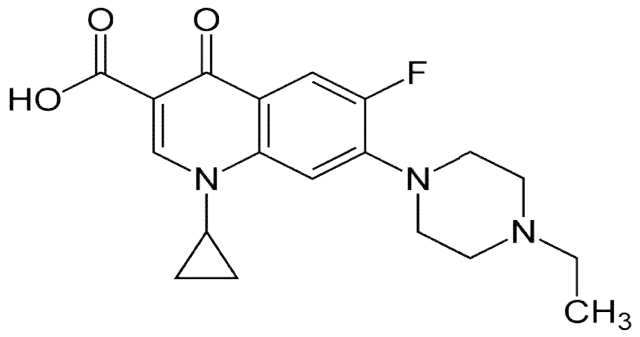


Figure 1. Structure of enrofloxacin

Chemicals and Reagents

Analytically pure sample of Enrofloxacin with purities greater than 99% was obtained as gift sample from Chandra labs, Hyderabad, India and tablet formulation [BAYTRIL] was procured from Apollo pharmacy, Hyderabad, India with label claim of 50mg. phosphate buffer was obtained from SD Fine chemicals (Hyderabad, India). 0.22µm nylon membrane filters were purchased from Spincotech Private Limited, Hyderabad, India.

Preliminary Solubility Studies:

Solubility studies were explored for Enrofloxacin in various solvents ranging pH of 1 to 7.5.

- **Distilled water:** 1mg of drug was added to 10ml of distilled water and found to be partially soluble even after sonication. Similar solubility procedure was followed using other solvents.
- **Preparation of pH 6.8 buffer as per USP:** To 50ml of mono basic potassium phosphate solution (0.2M, 22.7g/L) in a 200ml volumetric flask, was added 22.4ml of 0.2M NaOH solution and later made up to the volume with distilled water, whose pH was checked. If desired pH was not achieved, solution was adjusted to desired pH using dilute phosphoric acid and sodium hydroxide solutions.
- **Preparation of pH 4.5 buffer as per USP:** 2.99gm of sodium acetate in 1000ml volumetric flask was taken and then was added 14 ml 2N acetic acid solution which was finally made to the volume using water, whose pH was checked. If desired pH was not achieved, solution was adjusted to desired pH using dilute acetic acid and sodium hydroxide solutions. Enrofloxacin was found to be freely soluble and hence was used as dissolution media for developing method.
- **Preparation of pH 7.5 Buffer as per USP:** To 50ml of mono basic potassium phosphate solution (0.2M, 22.7g/L) in a 200ml volumetric flask, was added 37ml of 0.2M NaOH solution and made up the volume using distilled water, whose pH was checked. If desired pH was not achieved, solution was adjusted to desired pH using dilute phosphoric acid and sodium hydroxide solutions.
- **0.1N HCl:** 8.33 ml of concentrated HCl was made up to 1000ml using distilled water.

Preparation of Stock and Working Standard Solution

10mg of Enrofloxacin was accurately weighed and taken in 100ml clean and dry volumetric flask containing 80ml of solvent (phosphate buffer pH 4.5)and then the solution was made up to the mark using the solvent. This is considered as standard stock solution (100µg/ml). 0.5ml of the stock solution was pipetted out and made up to 10 ml to get a concentration 5µg/ml, treated as working standard, 100% target concentration for which UV spectrum was recorded (Figure 2). Suitable wavelength for the analysis was determined by recording UV spectrum in the range of 200-400 nm for 5µg/ml of Enrofloxacin standard as above and λ max was found to be 276nm and hence 276nm was chosen for the analysis.

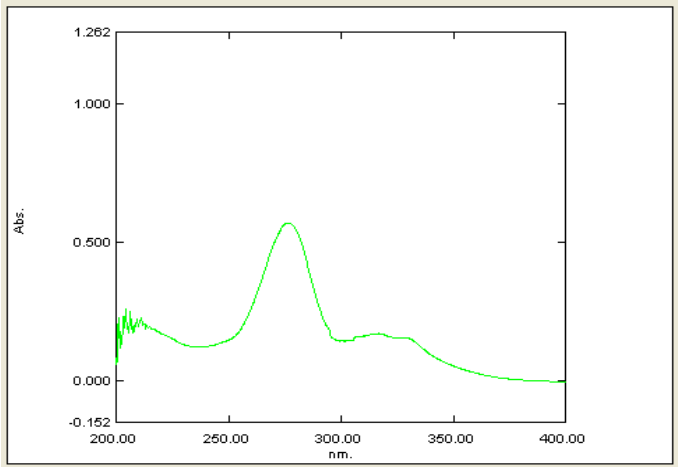


Figure 2. UV spectrum of standard (enrofloxacin)

Stability Studies: Both standard and sample were studied for stability by UV spectrophotometer at concentration of 5µg/ml and found to be minimum stable for 6 hours at room temperature as percentage degradation was within 2% and accordingly concluded to use this solvent for dissolution studies.

Dissolution Method Conditions: The optimized dissolution method includes the following keeping the acceptance criteria for % drug release (Q value) as greater than 85% at dissolution sampling point (Q point), 30min. Dissolution media volume was considered based on sink conditions where in dissolution media volume should be at least 3 times of saturation volume of the dose in the formulation

Rpm : 100
Dissolution medium: phosphate buffer pH 4.5
Dissolution media volume: 500mL
Apparatus: USP Type 2 (Paddle)
Sampling time point (Q point): 30 min
Sampling volume: 10ml
Temperature: 37±0.5ºC
Working concentration of standard: 5µg/ml
Working concentration of sample: 5µg/ml
Detection wavelength: 276nm

Preparation of Stock and Working Sample Solution

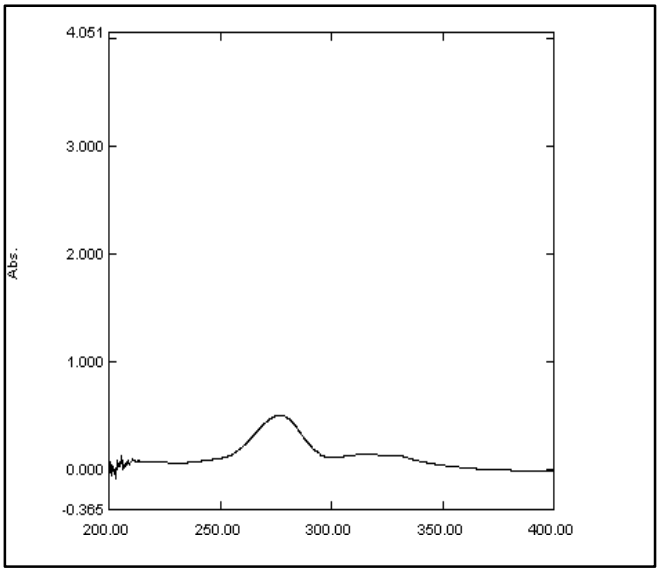
One tablet (dose:50mg) was studied under above dissolution conditions for 30 minutes and dissolution sample volume of 10ml was sampled out and later filtered through 0.22µm nylon filter. First few ml of the filtrate was discarded and then from

the filtrate (stock solution of sample), 0.5ml was pipetted out and made up to 10ml using phosphate buffer pH 4.5, to get working sample solution concentration equivalent to 5µg/ml, 100% target concentration as that of standard. UV spectrum of this solution was recorded which is shown in Figure3.

Absorbance of sample x Concentration of standard X100 /Average absorbance of standard x Concentration of sample

Method Validation

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. UV spectrophotometric dissolution method developed was validated according to International Conference on Harmonization (ICH) guidelines. The method was validated for the parameters like specificity, linearity, accuracy, system precision, intra-day precision and inter-day precision / intermediate precision/ruggedness.



% Drug release (Q value) was calculated using the formula as below:

Figure 3. Spectrum of sample

Precision

System Precision

Six replicate recording of absorbance at 276 nm of standard solution at working concentration showed % RSD (Relative Standard Deviation) less than 2, which indicates method is system precise. System precision results are tabulated below (Table 1).

Table 1. System precision results

N	abs
1	0.507
2	0.507
3	0.506
4	0.506
5	0.506
6	0.505
Average	0.506167
STDEV	0.000753
%RSD	0.14872

Table 2. Intraday Precision Results of enrofloxacin

n	abs	% Drug release
1	0.484	95.65217
2	0.48	94.67456
3	0.476	94.07115
4	0.483	95.45455
5	0.481	95.05929
6	0.471	93.26733
Average	0.479167	94.69651
STDEV	0.004875	0.900405
%RSD	1.017414	0.950832

Intermediate Precision (Inter day Precision/Ruggedness)

Dissolution studies were performed on six tablets by different analysts on two consecutive days and % RSD of percentage drug release was calculated and was found to be less than 2, which indicate the method developed is inter day precise/rugged (Table 3).

Table 3. Intermediate Precision / Ruggedness results of Enrofloxacin

n	day 1	day 2
1	95.65	95
2	94.67	93.43
3	94.07	94.82
4	95.45	95.8
5	95.05	94.035
6	93.26	95.4
Average	94.69	94.75
STDEV	0.9	0.876
%RSD	0.95	0.924

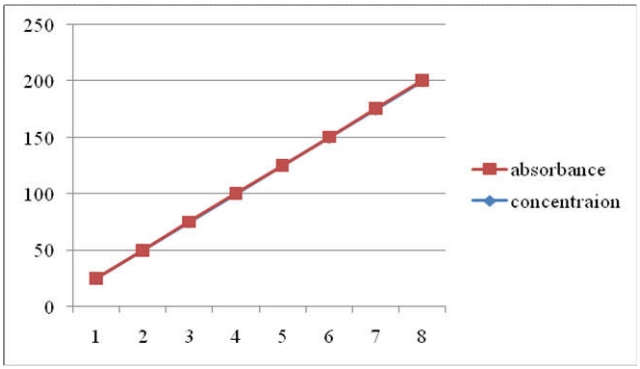


Fig. 4. Calibration of Enrofloxacin

Table 4. Calibration Data for enrofloxacin

n	% Level	Dilution	Concentration	absorbance
1	25	1.25-10	1.25	0.21
2	50	2.5-10	2.5	0.294
3	75	3.75-10	3.75	0.397
4	100	05-10	5	0.499
5	125	6.25-10	6.25	0.596
6	150	7.5-10	7.5	0.713
7	175	8.75-10	8.75	0.813
8	200	10	10	0.915
RSQ (r2)				0.999151741
SLOPE				0.081666667
INTERCEPT				0.09525

Linearity

Standard solutions of Enrofloxacin at different concentrations level (25%, 50%, 75%, 100%, 125%, 150%, 175% and 200%) were prepared. Calibration curve (Figure 4) was constructed by plotting the concentration of drug versus absorbance at 276

nm. The results show an excellent linear relationship between absorbance and concentration of drug within the concentration range of1.25-10µg/ml (Table 4). The correlation coefficient was found to be 0.999, which meet the method validation acceptance criteria and hence the method is said to be linear in the range of 1.25-10µg/ml.

Table 5. Results of Accuracy Studies for Enrofloxacin

Concentration	Absorbance	%recovery	Mean recovery
50	0.293	99.65986395	98.97959184
	0.292	99.31972789	
	0.288	97.95918367	
100	0.486	97.39478958	97.92919172
	0.491	98.39679359	
	0.489	97.99599198	
150	0.71	99.57924264	100.8415147
	0.729	102.2440393	
	0.718	100.7012623	

Table 6. Optical Characteristics and Validation Parameters of Enrofloxacin

Detection wavelength (nm)	276
Beer's Law limits (µg/ml)	1.25-10
Regression equation (y = mx+c)	y=0.08166x+0.09525
Correlation coefficient	0.999
Slope (m)	0.08166
Intercept (c)	0.09525
(% RSD) System precision	0.148
(% RSD) Intra-day precision	0.950
(% RSD) Inter-day precision	0.924
Accuracy (% Mean Recovery)	
50 % Level	98.97
100 % Level	97.92
150 % Level	100.84

Accuracy

Accuracy was determined by means of recovery experiments by the determination of % mean recovery of dissolution sample by absolute method at three different levels 50, 100% and 150%. At each level, three determinations were performed. Table 5 represents percent % mean recovery**.** Individual recovery and % mean recovery was found to be greater than 85% at 30 minutes, which indicates good recovery values and hence the accuracy of the method developed. Table 6 summarizes the validation parameters about the developed dissolution method.

Specificity

Blank (phosphate buffer pH 4.5) had zero absorbance at all wavelengths from 200-400nm while standard solution exhibited UV spectrum, hence the method is said to be specific for the analyte of interest.

Conclusion

A simple dissolution method by UV spectrophotometry method was developed and validated for the estimation of Enrofloxacinin rapidly immediate release tablets as per ICH guidelines. The optimized method uses phosphate buffer Ph4.5 as a solvent and dissolution medium, and detection wavelength of 276 nm. The developed method resulted in Enrofloxacin exhibiting linearity in the range 1.25-5µg/ml. System precision and intra-day precision are exemplified by relative standard deviation of 0.148% and 0.950% respectively. Method was found to be rugged as precision was found to be 0.924%. Accordingly it is concluded that the developed dissolution method by UV spectrophotometry is simple, accurate, precise, linear and rugged and therefore the method can be employed for the routine dissolution analysis of Enrofloxacin tablets in various pharmaceutical industries.

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