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Development and Validation for Simultaneous Estimation of Ciprofloxacin HCl, Doxycycline and Phenazopyridine HCl in Combined Dosage Form by U.V Method

Gajanan Jalindar Chavan[1*], Swapnali Roshan Charya[2], Ioan Nuw Baris[3], Sachin Dhondiram Patil[1]

1. *R & D Manager at Genpharma International Pvt.Ltd Pune.*
2. *R & D Officer at Genpharma International Pvt.Ltd.*
3. *Managing Director of Genpharma International Pvt.Ltd Pune.*

ABSTRACT

A simple, sensitive, accurate and precise simultaneous UV spectrophotometric method has been developed for the estimation of Ciprofloxacin HCL, Doxycycline Hyclate and Phenazopyridine HCL in tablet dosage form .The absorption maxima of the drugs were found to be 277, 273 and 392 nm for Ciprofloxacin HCL, Doxycycline Hyclate and Phenazopyridine HCL respectively, in water, using a Shimadzu UV–Visible spectrophotometer (model UV-1800). Ciprofloxacin HCL, Doxycycline Hyclate and Phenazopyridine HCL in obeyed Beer's law in the concentration range of 2-10 µg ml-1, 2-10 µg ml-1 and 2-10 µg ml-1 respectively. The correlation coefficient was found to be 0.999, 0.999, and 0.999 for Ciprofloxacin HCL, Doxycycline Hyclate and Phenazopyridine HCL respectively .The method was validated for various parameters according to ICH guidelines. The low relative standard deviation values indicate good precision and high recovery values indicate accuracy of the proposed method. Assay results were in good agreement with label claim.

Keywords: Ciprofloxacin HCL (CIPRO) , Doxycycline Hyclate(DOXY) and Phenazopyridine HCL(PHENA), UV spectrophotometric method, simultaneous equation method.

*Corresponding Author Email: gajanan8.10@gmail.com
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INTRODUCTION

Ciprofloxacin Hcl, Doxycycline and Phenazopyridine are available in tablet dosage form in the Ratio of 500mg, 100mg, 50mg Respectively . Chemically (ciprofloxacin hydrochloride) fluoroquinolone, is the monohydrochloride monohydrate salt of 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid. It is a faintly yellowish to light yellow crystalline substance with a molecular weight of 385.8. Its empirical formula is $C_{17}H_{18}FN_3O_3$•HCl•H_2O broad spectrum antimicrobial. Doxycycline hyclate is [4S(4aR,5S,5aR,6R,12aS)]-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro3,5,10,12,12a-pentahydroxy-6methyl-1,11-deoxonapthtacene-2-carboxamide

monohydrochloride, Doxycycline is a tetracycline antibiotic. It fights bacteria in the body. Doxycycline is used to treat many different bacterial infections, such as urinary tract infections, acne, gonorrhea, and chlamydia, periodontitis (gum disease), and others.phenazopyridine hydrochloride is chemically designated 2,6-Pyridinediamine, 3-(phenylazo), monohydrochloride. It is a urinary tract analgesic agent for oral administration Literature survey reveals, UV, HPLC methods for analysis of ciprofloxacin Hcl as single and combined dosage forms with other drugs and UV, HPLC methods for analysis of Doxycycline and Phenazopiridine as single component systems. There are no reported methods for analysis of this three drugs in combination. This paper presents simple, rapid, accurate and economical methods for simultaneous analysis of Ciprofloxacin HCL Doxycycline and Phenazopyridine HCL in combined tablet dosage form.

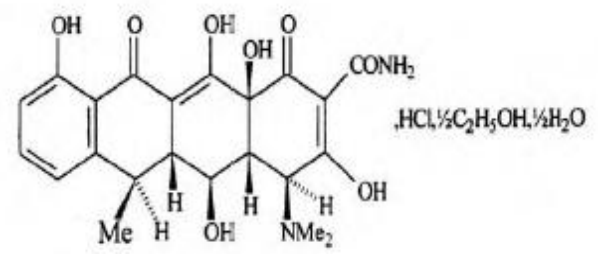

Figure 1a: Structure of Ciprofloxacin HCL **Figure 1a: Structure of Doxycycline Hyclate**

Figure 1c: Structure of Phenazopyridine

MATERIALS AND METHODS:

Procurement of drug samples and formulation:

Pure drug sample of Ciprofloxacin HCL with % purity 99.27, Doxycycline Hyclate 99.23

Phenazopyridine HCl with % purity 99.50, supplied as gift samples by Genpharma International Pvt. Ltd. were used without further purification. Tablet formulation Cephadox manufactured Genpharma International Pvt. Ltd. containing CIPRO 500 mg and DOXY100 mg and PHENA 50mg per tablet were purchased from local market and were used for analysis.

Reagents and chemicals used:

Double distilled water were used throughout the study. All the solvents and reagents used were purchased from LOBA Chem Pvt. Ltd., Mumbai. Double distilled water and placebo tablet were made at Lab scale only.

Instruments used:

An UV-Visible double beam spectrophotometer (Varian Cary 100) with 10 mm matched quartz cells was used. All weighing were done on electronic balance (Model Shimadzu AUW-220D). Ultrasonicator (Model 5.5 150H) was used for sample solution preparation.

Other Instruments:

1. Ultrasonicator : Ultrasonics
2. Water Purifier : Eco-still MARK 2000 DDQ
3. Magnetic Stirrer : Remi 2 MLH

Selection of solvent and wavelength:

Solubility of CIPRO, DOXY and PHENA was checked in solvents like water and methanol. UV spectrums of the three drugs in these solutions were recorded. The absorbance of the three drugs was found maximum in l solvent compared to other solvents and three wavelengths 278, 272 and 392 nm (Figure) were selected which are the λmax of CIPRO, DOXY and PHENA respectively.

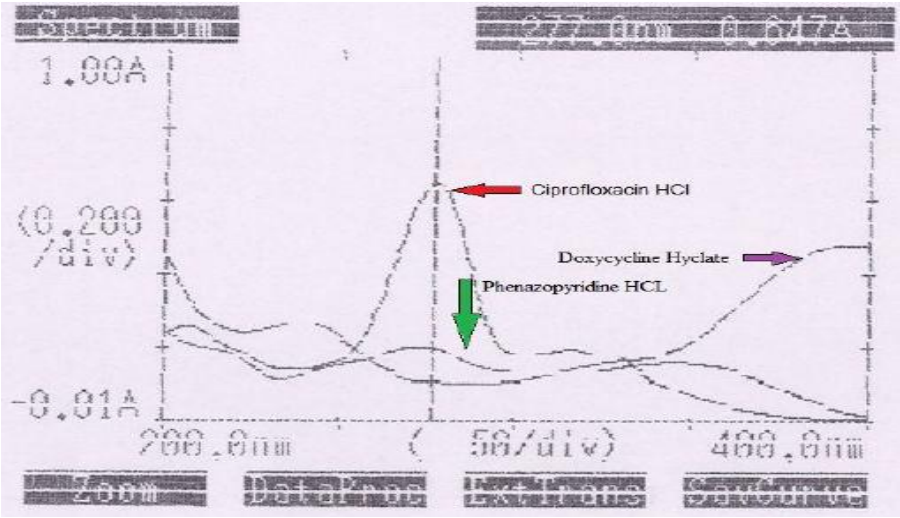

Figure 2: Overlay spectra of CIPRO, DOXY and PHENA showing selected wavelength

EXPERIMENTAL PROCEDURES:

Preparation Standard Stock Solutions: CIPRO, DOXY and PHENA (10 mg each) were separately weighed and transferred to 100 ml volumetric flask and all the three drugs were dissolved in Water to get a concentration of 100 µg ml-1.

Application of Simultaneous equation method:

In quantitative estimation of three components by Simultaneous equation method, three wavelengths i.e., CIPRO, DOXY and PHENA were selected as their respective λmax from the overlain spectrum, at which three drugs have maximum absorbance. The concentrations of three drugs in the mixture can be calculated using the following equations.

$$C_{CIPRO} = \frac{(A1\,(ay2az3-az2ay3) - ay1\,(A2az3-az2A3) + az1\,(A2ay3-ay2A3)}{ax1(ay2az3-az2ay3) - ay1(ax2az3-az2ax3) + az1(ax2ay3-ay2ax3)}$$

$$C_{DOXY} = \frac{(ax1(A2az3-az2A3) - A1(ax2az3-az2ax3) + az1(ax2A3-A2ax3)}{ax1\,(ay2az3-az2ay3) - ay1\,(ax2az3-az2ax3) + az1\,(ax2ay3-ay2ax3)}$$

$$C_{PHENA} = \frac{(ax1(ay2A3-A2ay3) - ay1(ax2A3-A2ax3) + A1(ax2ay3-ay2ax3)}{ax1\,(ay2az3-az2ay3) - ay1\,(ax2az3-az2ax3) + az1(ax2ay3-ay2ax3)}$$

Where, CCIPRO, CDOXY and CPHENA are the concentrations of CIPRO, DOXY and PHENA respectively in mixture and in sample solutions. A1, A2 and A3 are the absorbances of sample at 277, 273 and 392 nm, respectively, ax1, ax2 and ax3 are the absorptivity of CIPRO at 277, 273 and 392 nm respectively, ay1, ay2 and ay3 are the absorptivity of DOXY at 277, 273 and 392 nm respectively, az1, az2 and az3 are the absorptivity of PHENA at 277, 273 and 392 nm, respectively.

Analysis of marketed formulation:

For the analysis, 20 tablets were weighed and their average weight was determined. The tablets were then crushed to fine powder and powder equivalent to weight of one tablet was transferred to 100 ml volumetric flask and dissolved in 50 ml of Water for 10 min with vigorous shaking. Finally, the volume was made up to the mark with water . The solution was then filtered through whatmann filter paper. From this solution, 1 ml was pipette out into a 10 ml volumetric flask and diluted with methanol up to the mark. From this solution, 0.4 ml was transferred into a 10 ml volumetric flask and diluted with methanol up to the mark. The absorbance of the above solution was measured at 277, 273 and 392 nm. The concentration of each analyte was determined using the simultaneous equation.

RESULT AND DISCUSSION:

The analytical method was validated with respect to parameters such as linearity, precision, limit

of detection (LOD), limit of quantitation (LOQ) and accuracy.

Linearity:

Preparation of Calibration Curve and Linearity studies:

The individual standard solutions of these drugs containing 2-10 µg/ml were prepared by serial dilutions of standard stock solutions in water. Individual standard solutions were scanned using Water as blank. Instrument response at 277 nm and 273 nm and 292 nm was measured for CIPRO, DOXY and PHENA respectively, and used to prepare calibration curve. Six replicates of five individual standard solutions were used to prepare calibration curve.

Table 1: Calibration curve of CIPRO (n = 5) at 277.01 nm.

Sr. No.	Concentration (µg/ml)	Absorbance
1	0.0	0.155
2	2.0	0.305
3	4.0	0.456
4	6.0	0.603
5	8.0	0.759
6	10.0	0.909

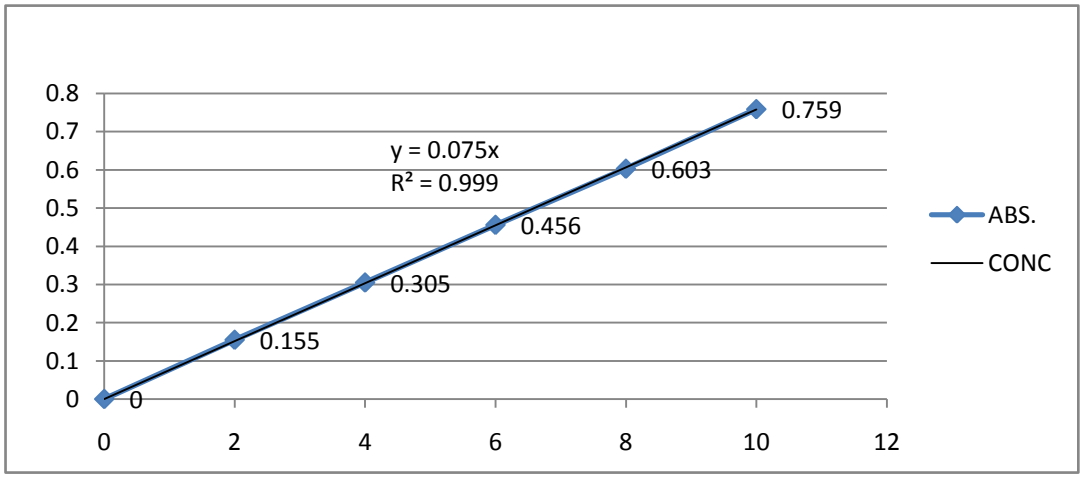

Fig.3: Standard calibration curve of CIPRO

Table 2: Calibration curve of DOXY (n = 5) at 273.0 nm.

Sr. No.	Concentration (µg/ml)	Absorbance
1	0.0	0.00
2	2.0	0.079
3	4.0	0.145
4	6.0	0.215
5	8.0	0.286
6	10.0	0.358

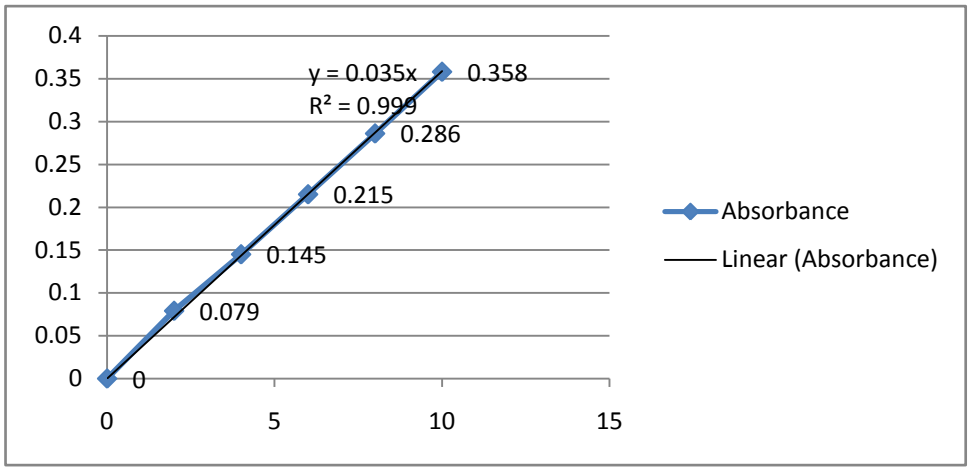

Fig.4: Standard calibration curve of DOXY

Table 3: Calibration curve of PHENA (n = 5) at 392.0 nm.

Sr. No.	Concentration (µg/ml)	Absorbance
1	0.0	0.000
2	2.0	0.064
3	4.0	0.123
4	6.0	0.189
5	8.0	0.239
6	10.0	0.307

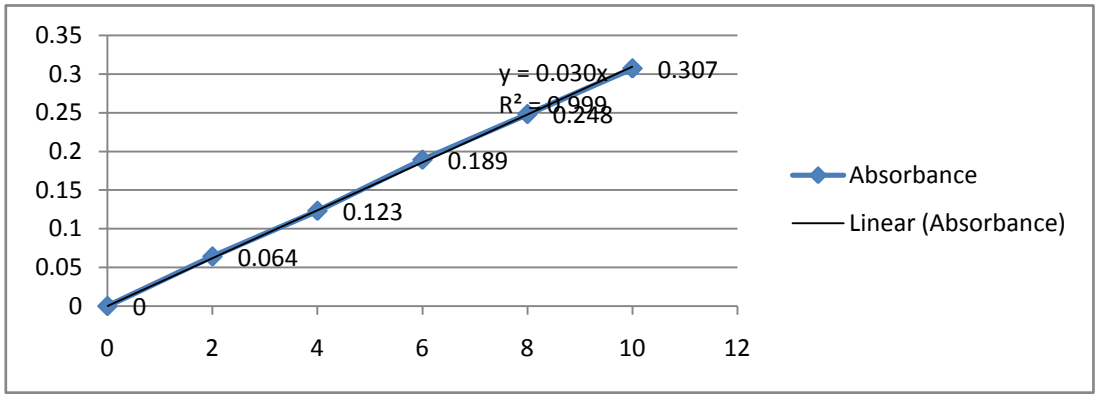

Fig.5: Standard calibration curve of PHENA

Precision:

The precision or repeatability was studied by six replicate analysis of tablet solutions containing 20 µg/ml of CIPRO, DOXY and PHENA respectively. The precision was also studied in terms of intra-day changes in absorbance of drug solution on the same day and on three different days. The intra-day precision of the developed method was determined by preparing the tablet samples of the same batch in nine determinations with three concentrations and three replicate each on same day. The inter-day precision was also determined by assaying the tablets in triplicate per

day for consecutive 3 days. The intra-day and inter-day variation was calculated in terms of percentage relative standard deviation. Precision of analyst was determined by repeating the method by another analyst working in the lab.

Table 5: Inter Intra day precision of CIPRO, DOXY and PHENA

Sr,No	Drug	Conc. (µg/ml)	Measured concentration (µg/ml), % R.S.D	
			Intra day	Inter day
1	CIPRO	20	0.346	0.389
2	DOXY	20	0.186	0.215
3	PHENA	20	0.278	0.345

Method Sensitivity (LOD and LOQ):

The Values of LOD and LOQ were calculated by using σ (standard Deviation of response) and b (Slope of the calibration curve) and by using equations,

LOD = (3.3 x σ)/ b and

LOQ = (10 x σ)/ b.

A. Limit of Detection (LOD)

Limit of detection was found to be:

CIPRO : 0.317 µg/ml

DOXY : 0.229 µg/ml

PHENA : 0.173 µg/ml

B. Limit of Quantification (LOQ)

Limit of quantification was found to be:

CIPRO : 0.303 µg/ml

DOXY : 0.216 µg/ml

PHENA : 0.162 µg/ml

The LOD and LOQ values of SIL and DAP indicate that the method is sensitive.

Accuracy:

The results of recovery studies at various levels shows that the recovery is between 98.58 to 100.08 % (Ideally should be between 98-102%). It indicates that there is no interference in the analysis of the drug from the excipients in the tablet formulation. The results of recovery studies of the marketed formulation are shown in the following Table-6.

Table 6: Results of the recovery analysis of CIPRO, DOXY and PHENA (n=3)

Compound	Recovery Level (%)	Recovery (%)	R.S.D (%)
CIPRO	50	99.16 %	0.63
	100	98.58 %	1.02
	150	98.55 %	0.48
DOXY	50	99.22 %	1.35
	100	99.07 %	1.41
	150	100.08 %	0.65
PHENA	50	99.12 %	1.35
	100	99.09 %	1.16
	150	100.10 %	0.56

Assay :

The tablets, Actinex™, purchased from local market, were analysed and the results were obtained in the range of 99.76-101.32 % compared to the label claim. The results of analysis of marketed formulation are shown in the following Table-7.

Table 7: Analysis of tablet formulation

Sr. No.	Label Claim (mg/tab)			Amount found (mg/tab)			% of Label claim determined		
	CIPRO	DOXY	PHENA	CIPRO	DOXY	PHENA	CIPRO	DOXY	PHENA
1	500	100	50	500.02	100.01	50.02	500.03	100.26	50.03
2	500	100	50	500.10	100.06	50.05	500.23	100.01	50.14
3	500	100	50	500.16	100.05	50.04	500.13	100.03	50.01
4	500	100	50	500.03	100.15	50.12	500.02	100.02	50.01
5	500	100	50	500.03	100.01	50.01	500.15	100.05	50.13
6	500	100	50	500.15	100.14	50.10	500.15	100.02	50.10
			Mean	500.12	30.05	50.06	500.16	100.06	50.06
			Standard Deviation	0.05	0.12	0.16	0.06	0.14	0.18
			% RSD	0.10	0.43	0.48	0.11	0.46	0.51

CONCLUSION:

The developed UV spectrophotometric method is simple, precise, accurate, linear, reproducible and repeatable for the estimation of CIPRO, DOXY and PHENA in pharmaceutical dosage forms without any interference from the excipients. It can be successfully applied for the routine analysis of all the three drugs in pharmaceutical dosage forms.

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