JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

Absorption Correction Method for Simultaneous Estimation of Phenazopyridine HCl and Ciprofloxacin HCl in Combined Tablet Dosage Form

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ABSTRACT:

A new, simple, precise, accurate and sensitive UV - Spectrophotometric absorption correction method has been developed for simultaneous determination of Phenazopyridine HCl and Ciprofloxacin HClin combined tablet dosage form. Methanol was used as solvent. Absorbance correction method was based on the property of additivity of absorbances. The two wavelengths on Phenazopyridine HClcurve were found out, which were 279 and 413 nm. At 413 nm, Phenazopyridine HClshowed some absorbance while Ciprofloxacin HClshowed zero absorbance. Both the drugs gave absorbance at 279 nm. The method involved solving of an equation based on measurement of absorbance at two wavelengths 279 and 413 nm. The determinations were made at 279 nm for Phenazopyridine HCl and Ciprofloxacin HCl and 413 nm for Phenazopyridine HClover the concentration range of 2-10 µg/ml for Phenazopyridine HCl and 2.5-12.5 µg/ml for Ciprofloxacin HClwith mean recovery of 99.56% and 100.63% for Phenazopyridine HCl and Ciprofloxacin HCl, respectively by absorbance correction method. This method was found to be simple, sensitive, accurate, precise, reproducible and economical and can be applicable for the simultaneous determination of Phenazopyridine HCl and Ciprofloxacin HClin combined dosage form.

KEYWORDS: Phenazopyridine HCl, Ciprofloxacin HCl, Absorbance correction method, Validation, Combined dosage form

Article history:
Received 19 Mar 2014
Accepted 03 April 2014
Available online 13 April 2014

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INTRODUCTION:

Ciprofloxacin HCl (CIPRO) Tablets and Ciprofloxacin Oral Suspension are synthetic broad spectrum antimicrobial agents for oral administration. Ciprofloxacin hydrochloride, a fluoroquinolone, is the monohydrochloride monohydrate salt of 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid[1,2]. It is official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP) and United States Pharmacopoeia (USP). IP[3], BP[4] and USP[5] describe HPLC method for its estimation. Various methods like UV spectrophotometry[6,7], RP-HPLC[8,9], spectrophotometric method for simultaneous determination of CIPRO with other drug and RP-HPLC method for simultaneous determination of CIPRO with other drug are reported in literature for estimation of CIPRO in pharmaceutical dosage forms.Phenazopyridine HCl(PHENA) is chemically (3-(Phenylazo)-2,6–pyridinediaminemonohydrochloride). It is a urinary tract analgesic agent for oral administration[1,2]. Phenazopyridine HCl is official in United States Pharmacopoeia (USP). USP[5] describe HPLC method for its estimation. Various methods like spectrophotometric[10],HPLC[11-13] and GC-MS[14], Electroanalyrical method[15]method for simultaneous estimation of PHENA with other drug, and RPHPLC method for simultaneous estimation of PHENA with other drug for the determination of PHENA are reported in literature for estimation of PHENA in pharmaceutical dosage forms. The combined dosage forms of CIPRO and PHENA are available in the market for the treatment of Urinary Tract Infection. Literature survey reveals the simple

the simple spectroscopic method for determination of CIPRO and PHENA in combined dosage form based on Absorbance correction method using methanol as a solvent. The present manuscript describes alternative simple, sensitive, accurate, precise, reproducible, and economical absorbance correction method for simultaneous estimation of CIPRO and PHENA in combined dosage form.

MATERIAL AND METHODS

Instruments

Spectrophotometric measurements were performed on Shimadzu UV –visible double beam spectrophotometer (Model- 1800). All weighing were done on electronic analytical balance (Wensar Dab220).

Chemicals and Reagents

CIPRO and PHENA bulk powder was obtained by Shris pharmaceuticals Pvt. Ltd., Hyderabad, AP, India. The commercial fixed dose combinationUTIstat tablet was procured from the local market. All other chemicals used were of analytical grade. Caliberated glasswares were employed throughout the work.

Preparation of Standard Stock Solution

Ciprofloxacin HCl standard stock solution (100 µg/ml): Accurately weighed 10 mg of Ciprofloxacin HCl was taken in 100 ml volumetric flask and diluted with methanol up to the mark.

Phenazopyridine HCl standard stock solution (100 µg/ml) : Accurately weighed 10 mg of Phenazopyridine HCl was taken in 100 ml volumetric flask and diluted with methanol up to the mark.

Preparation of working standard solutions

Accurately measured standard solutions of Ciprofloxacin HCl (0.25, 0.5, 0.75, 1.0, 1.25 ml) and Phenazopyridine HCl(0.2, 0.4, 0.6, 0.8, 1.0 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with methanol to prepare 2.5, 5, 7.5, 10, 12.5 µg/ml and 2, 4, 6, 8, 10 µg/ml solutions of Ciprofloxacin HCl and Phenazopyridine HCl respectively.

Selection of Analytical Wavelength

By appropriate dilution of two standard drug solutions with methanol, solutions containing 10 µg/ml of Ciprofloxacin HCl and 10 µg/ml of Phenazopyridine HCl were scanned separately in the range of 200-800 nm. Overlain spectra[Fig 3] show 279 nm as the λmax of CIP HCl and 413 nm as the λmax of PHE HCl.

Assay

The absorbance of sample solution was measured at 279 nm and 279 nm and 413 nm and the amount of Ciprofloxacin HCl and Phenazopyridine HCl respectively were calculated through the simultaneous equation method by calibration curve of Ciprofloxacin HCl and Phenazopyridine HCl[Table 3]

Method Validation

Method validation was performed following ICH guidelines[16]. The proposed method has been extensively validated in terms of linearity, accuracyand precision, limit of detection and limit of quantification.

Linearity (Calibration curve)

Calibration curves[Fig 4,5,7-9] were plotted over a wide concentration range and the linear response was observed over a concentration range of 2.5-12.5 µg/ml for Ciprofloxacin HCl and 2-10 µg/ml for Phenazopyridine HCl. Accurately measured standard solutions of Ciprofloxacin HCl (0.25, 0.5, 0.75, 1.0, 1.25 ml) and Phenazopyridine HCl (0.2, 0.4, 0.6, 0.8, 1.0 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with Methanol, and the absorbance was measured(n=6) at 279 nm (λmax of Ciprofloxacin HCl) and at 279 nm and 413nm (λmax of Phenazopyridine HCl). The calibration curves were constructed by plotting absorbance vs. concentrations. The linear regression equation of Ciprofloxacin HCl[Fig 7]was y = 0.098x + 0.013 (R^2= 0.999) and Phenazopyridine HCl[Fig 8,9] at 279nm was y = 0.050x – 0.007 (R^2= 0.999) and at 413nm was y = 0.104x + 0.020(R^2= 0.998)

Accuracy

Accuracy[Table 2] was assessed by determination of the recovery of the method by addition of standard drug to the prequantified sample preparation at three different concentration levels 80 %, 100 % and 120 %, taking in to consideration percentage purity of added drug sample. The amounts of Ciprofloxacin HCl and Phenazopyridine HCl were estimated by applying obtained values to the respective regression line equations. Each concentration was analysed 3 times and average recovery were measured.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the method was verified as repeatability, intra-day, inter-day and reproducibility.

The precision of the method was checked by repeated measurement of absorbance of solution of (*n* = 6) of Ciprofloxacin HCl (7.5 µg/ml) and Phenazopyridine HCl (6 µg/ml) without changing the parameter of proposed method. %RSD was calculated.

Limit of Detection and Limit of Quantification

ICH guideline describes several approaches to determine the detection and quantification limits. These include visual evaluation, signal-to-noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and were calculated according to the 3.3 × (SD/Slope) and 10 × (SD/Slope) criteria, respectively; where SD is the standard deviation of y-intercept of regression line and S is the slop of the calibration curve.

RESULT AND DISCUSSION

A reliable absorption correction method was developed for simultaneous estimation of Ciprofloxacin HCl and Phenazopyridine HClin combined tablet dosage formby UV Spectrophotometry. Beers law was obeyed in concentration range of 2.5-12.5µg/ml for Ciprofloxacin HCl and 2-10µg/ml for Phenazopyridine HCl at 279 nm and 413 nmwavelengths, respectively. The correlation coefficient Ciprofloxacin HCl and Phenazopyridine HClwas found to be R^2= 0.999 and 0.999. The mean % recoveries were found to be in the range of 98.60-101.52% and 98.98-101.60%, respectively. Precision (% RSD) of Ciprofloxacin HCl and Phenazopyridine HCl was found to be 0.1-1.4 % and 0.5-1.3 %, respectively. The LOD and LOQ were 0.096µg/ml and 0.292µg/ml of Ciprofloxacin HCland 0.062µg/ml and 0.188µg/ml of Phenazopyridine HCl, respectively. The proposed method was precise, accurate and reproducible and acceptable recovery of the analytes, which can be applied for the analysis of Ciprofloxacin HCl and Phenazopyridine HCl in combined tablet dosage form[Table 1].

CONCLUSION

The results of our study indicate that the proposed UV spectroscopic method is simple, rapid, precise and accurate. The developed UV spectroscopic method was found suitable for determination of Ciprofloxacin HCl and Phenazopyridine HCl in combined tablet dosage form without any interference from the excipients. Statistical analysis proves that the method is repeatable and selective for the analysis of Ciprofloxacin HCl and Phenazopyridine HCl. It can therefore be conclude that use of the method can save time and money and it can be used in small laboratories with accurate and wide linear range.

ACKNOWLEDGEMENT

The authors are thankful to Dr. K. Pundarikakshudu, Director of L. J. Institute of Pharmacy, Ahmedabad, India for providing all the facilities to carry out the work.

The authors are thankful to Lincoln Pharmaceuticals Ltd., Ahmedabad, Gujarat, India for providing gift sample of Ciprofloxacin HCl and Phenazopyridine HCl for research.

Figure 1: Structure of Ciprofloxacin HCl

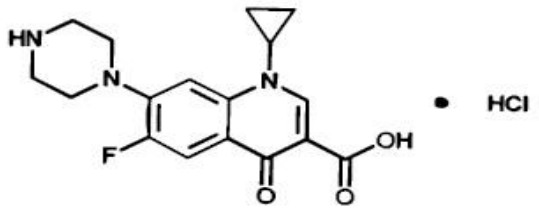

Figure 2: Structure of Phenazopyridine HCl

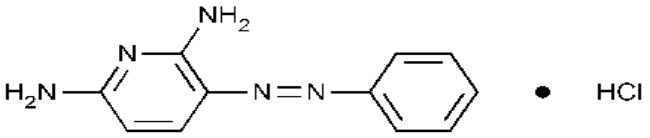

Table 1: Regression analysis data and summary of validation parameters for the proposed method

Validation Parameter	Ciprofloxacin HCl	Phenazopyridine HCl
Linearity		
Regression Equation	Y=0.098x +0.013	Y=0.050x -0.007
Regression Coefficient	R^2 = 0.999	R^2 = 0.999
Range	2.5-12.5	2-10
Accuracy (%Recovery)	98.60-101.52	98.98-101.60
Precision (%RSD)		
Repeatability	0.44	1.34
Intraday	0.1-0.4	0.6-1.3
Inter-day	0.1-0.4	0.5-1.0
LOD (µg /ml)	0.096	0.062
LOQ (µg /ml)	0.292	0.188
% Assay	99.56	100.63

Table 2: Recovery data of proposed method

Drug	Level (%)	Test amount (µg/ml)	Spiked STD amount (µg/ml)	Total amount recovered (µg/ml)	% Mean recovery ± SD. (n=3)
Ciprofloxacin HCl	80	5	4	9.02	100.28 ± 0.902
	100	5	5	9.94	99.47 ± 0.904
	120	5	6	11.05	100.57 ± 0.829
Phenazopyridine HCl	80	4	3.2	7.20	100.14 ± 0.926
	100	4	4	8.01	100.23 ± 1.058
	120	4	4.8	8.77	99.71 ± 0.560

Table 3:Analysis of Tablet

Active ingredient	Label claim	Test Concentration (µg/ml)	Amount found (µg/ml)	% Assay (n=3)
Ciprofloxacin HCl	250 mg	12.5	12.445	99.56
Phenazopyridine HCl	200 mg	10	10.063	100.63

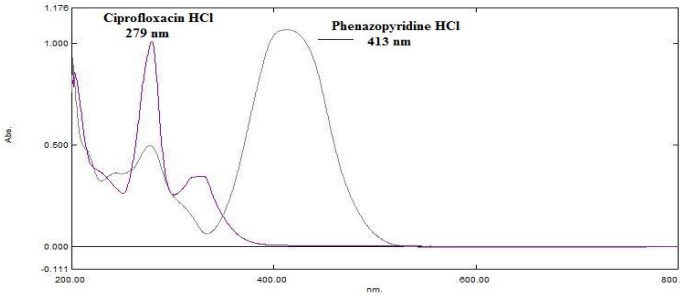

Figure 3: UV Spectrum for Ciprofloxacin HCl (10 µg/ml) at 279 nm and Phenazopyridine HCl (10 µg/ml) at 413 nm in methanol.

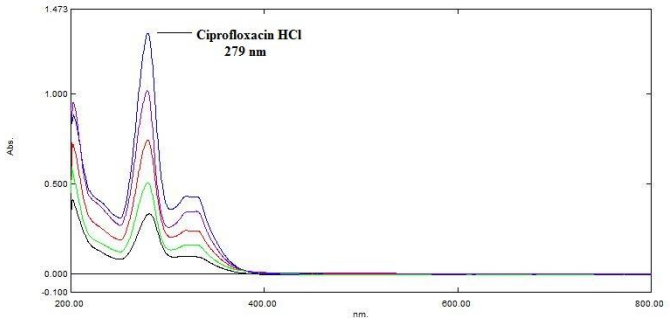

Figure 4: Overlain spectrum of Ciprofloxacin HCl (2.5-12.5 µg/ml) in methanol.

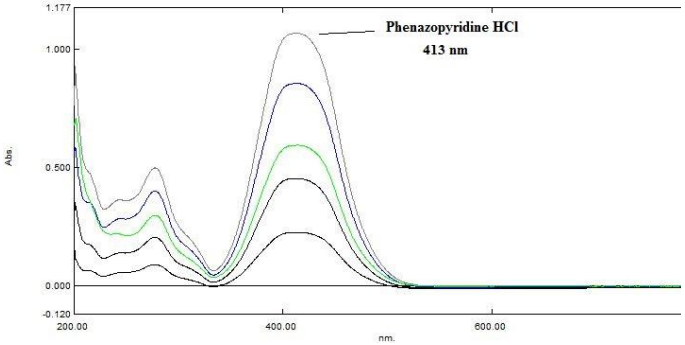

Figure 5: Overlain spectrum of Phenazopyridine HCl (2-10 µg/ml) in methanol.

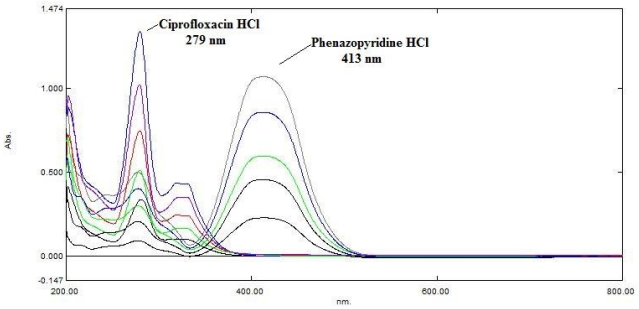

Figure 6: Overlain spectrum of Ciprofloxacin HCl (2.5-12.5 µg/ml) and Phenazopyridine HCl (2-10 µg/ml) in methanol.

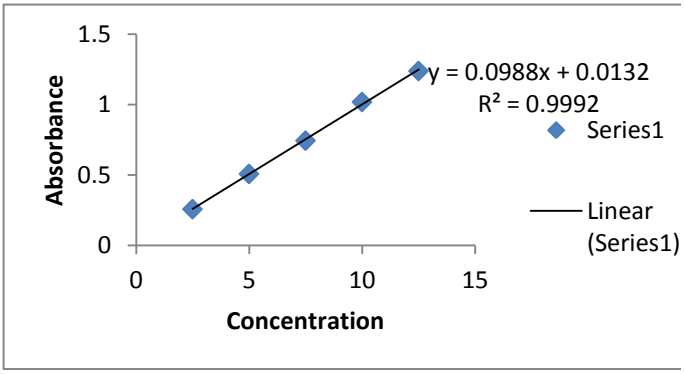

Figure 7: Calibration curve of Ciprofloxacin HCl at 279 nm in methanol.

Figure 8: Calibration curve of Phenazopyridine HCl at 279nm in methanol.

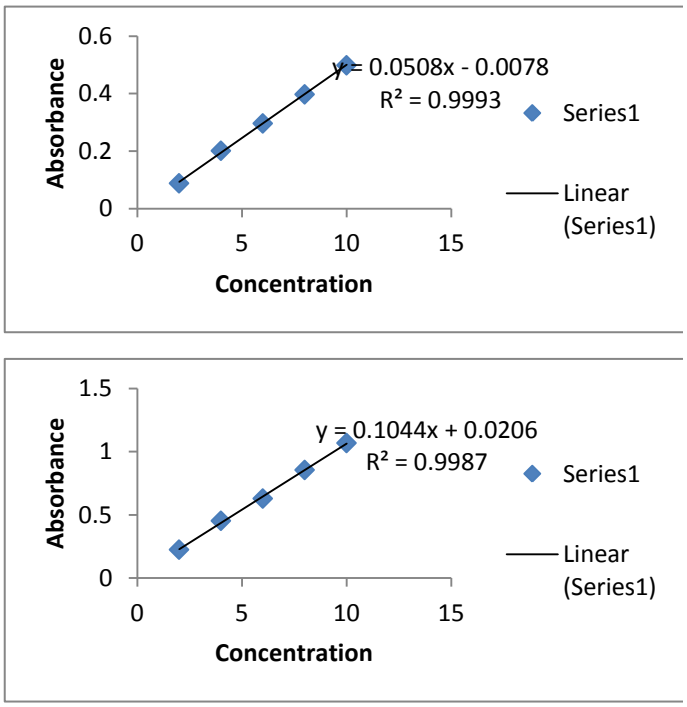

Figure 9: Calibration curve of Phenazopyridine HCl at 413nm in methanol.

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