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Dual Wavelength Spectrophotometric Method for Simultaneous Estimation of Ciprofloxacin and Phenylephrine Hydrochloride in Combined Dosage Form

PATEL PARESH U., PATEL HARSH B.*

Department of Pharmaceutical Quality Assurance, Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Kherva – 382711, Mehsana, Gujarat, India.

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Abstract:

A simple, accurate and precise dual wavelength spectrophotometric method was developed for simultaneous determination of Ciprofloxacin and Phenylephrine Hydrochloride in combined pharmaceutical dosage form. The principle for dual wavelength method is "the absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest". The wavelengths selected for determination of Ciprofloxacin were 272.09 nm and 305.85 nm, whereas, the wavelengths selected for determination of Phenylephrine Hydrochloride were 256.80 nm and 282.96 nm. 0.1 M NaOH was taken as a solvent. Regression analysis of Beer's plots showed good correlation in concentration range of 1-12 µg/ml for Ciprofloxacin and 5-30 µg/ml for Phenylephrine Hydrochloride. Accuracy of method was found between 99.93 - 101.65 %. The precision (intra-day, inter-day and repeatability) of method was found within limits (%CV<2). The proposed methods are simple, rapid, economic and accurate for routine simultaneous estimation of Ciprofloxacin and Phenylephrine Hydrochloride.

Keywords: Ciprofloxacin, Phenylephrine Hydrochloride, Dual wavelength, Linearity, Validation.

Introduction

Ciprofloxacin (CIP) is chemically 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid.[1] It is a synthetic antibiotic of the fluoroquinolone drug class.[2] It is a second-generation broad-spectrum antimicrobial carboxyfluoroquinoline. It is used for the treatment of the bacterial infections caused by susceptible organisms. It kills bacteria by interfering with the enzymes that cause DNA to rewind after being copied, which stops DNA and protein synthesis.[3] The bactericidal action of CIP results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair, strand supercoiling repair, and recombination. It is official in IP[4] BP[5] and USP.[6] HPLC[7, 8], HPTLC[9] and atomic absorption spectrometry[10] methods for estimation of CIP alone in pharmaceutical preparation have been reported.

Phenylephrine Hydrochloride (PEH) is chemically (R) -1-(3-hydroxyphenyl)-2-methylaminoethanol hydrochloride. PEH is α1-adrenoceptor agonist which stimulates postsynaptic alpha receptor cause vasoconstriction, systolic and diastolic pressure. It is indicated for nasal congestion, minor eye irritations and open angle glaucoma.[11, 12] It is official in IP[13], BP[14] and USP.[15] The pure drug is estimated by titration but the formulations are assayed by UV spectrophotometry and HPLC according to these official books. The estimation of PEH using simple UV spectroscopy, HPLC and HPTLC has been reported in combination with other drugs.[16-25] Colorimetry were developed on PEH present in formulation as single component.[26-29] Since no spectrophotometric method is reported for simultaneous estimation of CIP and PEH in combined dosage form therefore, in the present

work; a successful attempt has been made to estimate both these drugs simultaneously by Dual Wavelength Method.[30-32]

MATERIALS & METHODS

Appratus

1. A Double beam UV-visible spectrophotometer (Shimadzu, UV-1800, Japan) attached to a computer software UV prob 2.0, with a spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells.
2. Analytical balance (CP224S, Sartorius, Germany).
3. Ultrasonic cleaner (Frontline FS 4, Mumbai, India).
4. Corning volumetric flasks and pipettes of borosilicate glass were used in the study.

Reagents & Materials

1. Ciprofloxacin (CIP), Phenylephrine Hydrochloride (PEH) and Benzalkonium Chloride (BKC) were kindly supplied as gift samples from Medwin Pharmaceutical, Naroda GIDC, Ahmedabad.
2. The pharmaceutical formulation containing 0.3 % CIP, 0.01 % PEH and 0.01% BKC was kindly supplied from market.
3. AR grade Sodium Hydroxide (NaOH) (S.D. Fine Chemical Ltd., Mumbai, India).
4. Whatmann filterpaper No. 41 (Whatmann International Ltd., England).

Preparation of 0.1 M Sodium Hydroxide Solution

An accurately weighed 4 gm Sodium Hydroxide pallets were dissolved in 1000 ml distilled water.

Preparation of Standard Stock Solutions

Accurately weighed portion of CIP (10 mg) and PEH (10 mg) was transferred to separate 100 ml volumetric flasks and dissolved and diluted to the mark with 0.1 M NaOH to obtain standard solution having concentration 100 µg/ml.

Preparation of working standard solution

20 µg/ml of CIP solution was prepared by diluting 2 ml of standard stock solution to 10 ml with 0.1 M NaOH. 20 µg/ml of PEH solution was prepared by diluting 2 ml of stock solution to 10 ml using 0.1 M NaOH.

DUAL WAVELENGTH METHOD

The utility of dual wavelength data processing program is to calculate the unknown concentration of a component of interest present in a mixture containing both the components of interest and an unwanted interfering component by the mechanism of the absorbance difference between two points on the mixture spectra. This is directly proportional to the concentration of the component of interest, independent of the interfering components. The pre-requisite for dual wavelength method is the selection of two such wavelengths where the interfering component shows same absorbance whereas the component of interest shows significant difference in absorbance with concentration.

Study of overlain spectra and selection of wavelength

Solutions having 20 µg/ml of both drugs CIP and PEH were prepared respectively and scanned over the range of 200- 400 nm and the overlain spectra were observed for development of suitable method for analysis. The overlain spectra of CIP and PEH are shown in figure 3. From the overlay spectra two wavelengths 272.09 nm and 305.85 nm were selected as λ_1 and λ_2 for the estimation of CIP. PEH shows the same absorbance at these wavelengths. Similarly, wavelengths 256.80 nm and 282.96 nm were selected as λ_3 and λ_4 for estimation of PEH. For calibration curve, from the working standard solutions, appropriate dilutions in the range of 1-12 µg/ml and 5-30 µg/ml for CIP and PEH respectively were prepared and analyzed.

VALIDATION

The proposed methods were validated as per ICH guidelines. [33]

1. Linearity

Linearity was observed in a concentration range of 1-12 µg/ml and 5-30 µg/ml for CIP and PEH respectively. For the evaluation of the range, accurately measured standard working solution of CIP (0.1, 0.4, 0.6, 0.8, 1.0 and 1.2 ml) and PEH (0.5, 1.0, 1.5, 2.0, 2.5 and 3ml) was pipette out in to a separate series of 10 ml volumetric flasks. The volume was adjusted with 0.1 M NaOH and absorbance of all the solutions was measured at respective wavelengths for each drug separately.

2. Method Precision (Repeatability)

The precision of the instrument was checked by repeated scanning and measuring the absorbance of solutions (n=6) of CIP (6 µg/ml) and PEH (15 µg/ml) at respective wavelengths for each drug separately without changing the parameters of the dual wavelength method. The results are reported in terms of percentage relative standard deviation (%RSD).

3. Intermediate Precision (Reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of CIP and PEH (6, 8 and 12 µg/ml for CIP and 10, 15 and 20 µg/ml for PEH). The results are reported in terms of percentage relative standard deviation (%RSD).

4. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) of the method were calculated by using the following equations as per ICH guideline.

LOD= 3.3*σ/S

LOQ=10* σ /S

Where, σ = the standard deviation of the absorbance

And S = slope of the calibration curve.

5. Accuracy (% Recovery)

The accuracy of the method was determined by calculating recoveries of CIP and PEH by the standard addition method. Known amounts of standard solutions of CIP and PEH were added at 50%, 100% and 150% levels to prequantified sample solutions of CIP (3 µg/ml) and PEH (4 µg/ml).

ANALYSIS OF PHARMACEUTICAL FORMULATION

The solution (5 ml) containing 0.3 % CIP, 0.01% PEH and 0.01% BKC was transferred to 100 ml volumetric flask and diluted up to mark with 0.1 M NaOH to obtain solution having concentration 150 µg/ml of CIP and 5 µg/ml of PEH. Here concentration of PEH was very low thatswhy for assay of this formulation, 95 mg standard PEH was added to obtain solution having concentration 150 µg/ml of CIP and 100 µg/ml of PEH. This solution (0.4 ml) was taken in to a 10 ml volumetric flask and the volume was adjusted up to the mark with 0.1 M NaOH to get final concentration of CIP (6 µg/ml) and PEH (4 µg/ml). The concentration of both CIP and PEH were determined by measuring the absorbance of the sample solution at the selected wavelengths against 0.1 M NaOH in presence of 0.01% BKC standard as blank. Concentration of CIP and PEH in sample solution was determined by dual wavelength method. From these absorbance values, the concentration of CIP and PEH were determined using calibration graph. The absorbance was measured at the selected wavelengths and concentrations were determined. The analysis was done in triplicate.

RESULTS & DISCUSSION

Simple, precise and accurate Dual Wavelength Method was developed for the simultaneous estimation of CIP and PEH in combined dosage forms. The wavelengths used for Dual Wavelength Method. From the overlay spectra two wavelengths 272.09 nm and 305.85 nm were selected as λ_1 and λ_2 for the estimation of CIP. PEH shows the same absorbance at these wavelengths. Similarly, wavelengths 256.80 nm and 282.96 nm were selected as λ_3 and λ_4 for estimation of PEH. CIP shows the same absorbance at these wavelengths. Beer's law obeyed in concentration range 1-16 µg/ml for CIP and 5-30 µg/ml for PEH for Dual Wavelength Method. The commercial formulation containing CIP and PEH was analyzed by the proposed method. The percentage relative standard deviation for precision and accuracy was found to be low, which indicates that the method has considerable accuracy and precision. Standard calibration curves of CIP and PEH for Dual Wavelength Method were linear with correlation coefficient (r^2), slope and intercept 0.9966, 0.0666 and 0.0369; 0.9974, 0.0055 and 0.0111, respectively. For intraday precision, the method was repeated 3 times in a day and the average % RSD was found to be 0.21 for CIP and 0.45 for PEH. Similarly, for interday precision, the method was repeated on 3 different days and average % RSD was found to be 0.24 for CIP and 0.59 for PEH. These values confirm the intra and interday precision of the method. Accuracy of the method was confirmed by recovery studies on preanalyzed formulations. Recovery greater than 99% with the low standard deviation justifies the accuracy of the method. The results are in good agreement with the label claim. The proposed method is found to be simple, precise, accurate and sensitive and therefore, can be used as a quality control tool for the simultaneous estimation of both drug from their combined dosage form in quality control laboratory.

CONCLUSION

The proposed dual wavelength method gives accurate and precise results for determination of Ciprofloxacin and Phenylephrine Hydrochloride in marketed formulation without prior separation and is easily applied for routine analysis. The most striking feature of the dual wavelength method is its simplicity and rapidity. Method validation has been demonstrated by variety of tests for linearity, accuracy, LOD, LOQ and precision. The proposed method was successfully applied to determination of these drugs in pharmaceutical formulation.

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ABBREVIATIONS

CIP: Ciprofloxacin

PEH: Phenylephrine Hydrochloride

BKC: Benzalkonium Chloride

ICH: International Conference on Harmonization

RSD: Relative Standard Deviation
S.D: Standard Deviation
IP: Indian Pharmacopoeia
BP: British Pharmacopoeia
USP: United States Pharmacopoeia
UV: Ultraviolet
HPLC: High Performance Liquid Chromatography
HPTLC: High Performance Thin Layer Chromatography
LOD: Limit of Detection
LOQ: Limit of Quantification

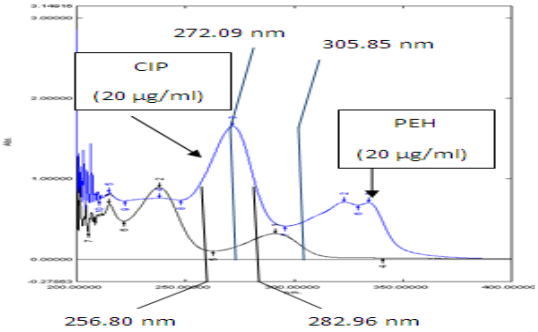

Figure 1: Overlain zero order spectra of CIP and PEH in 0.1 M NaOH

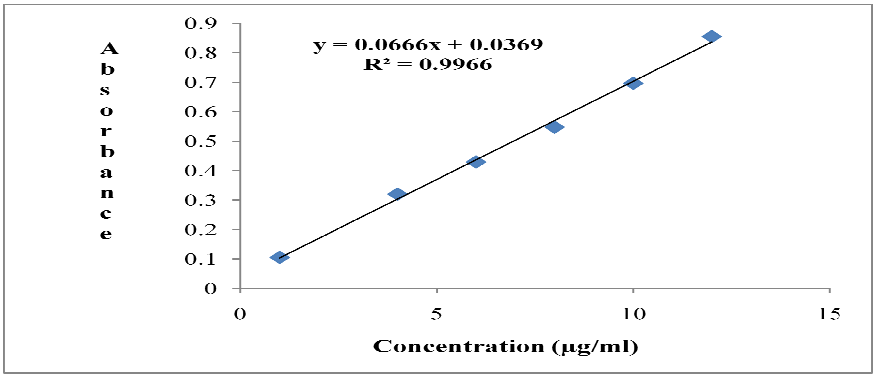

Figure 2: Calibration curve for CIP

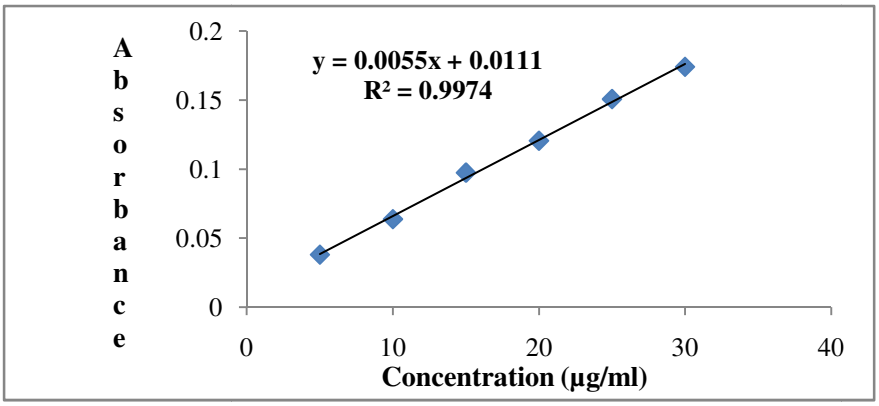

Figure 3: Calibration curve for PEH

Table 1 : Optimized method parameters for Dual Wavelength Spectrophotometry

Method Parameters	Optimized Parameters
Solvent	0.1 M NaOH
Scanning Range	200-400 nm
Scanning Speed	Fast
Analytical wavelength for determination of CIP	272.0 nm and 305.8 nm
Analytical wavelength for determination of PEH	256.8 nm and 282.9 nm

Table 2 : Recovery Data for CIP and PEH

Drug	Amount Present (µg/ml)	Amount Added (%)	%Recovery ± S.D. (n=3)
CIP	3	50	101.43 ± 1.41
	3	100	99.93 ± 1.15
	3	150	100.78 ± 1.25
PEH	4	50	101.65 ± 0.94
	4	100	100.86 ± 1.47
	4	150	101.03 ± 0.11

Table 3 : Analysis of CIP and PEH in formulation

Label Claim (%)		Amount Found (%)		% Label Claim ± S.D. (n=3)	
CIP	PEH	CIP	PEH	CIP	PEH
0.3	0.01	0.30685	0.01009	102.37 ± 0.46	101.35 ± 1.85

Table 4 : Validation Parameters for Dual Wavelength Method

Parameters	CIP	PEH
Beer's law limit	1-12	5-30
Regression Equation y= mx + c Slope Intercept	y = 0.0666x + 0.0369 0.0666 0.0369	y = 0.0055x + 0.0111 0.0055 0.0111
Correlation coefficien(r^2)	0.9966	0.9974
LOD (µg/ml)	0.11	1.16
LOQ (µg /ml)	0.34	3.52
Repeatability (% RSD ,n =6)	0.69	0.31
Precision (%RSD, n=3)		
Intraday	0.13-0.36	0.42-0.50
Interday	0.12-0.45	0.28-0.66
Accuracy ± S.D. (% Recovery, n=3)	100.71 ± 1.27	101.18 ± 1.14
Assay ± S.D. (n=3)	102.37 ± 0.46	101.35 ± 1.85

LOQ = Limit of quantification- LOD = Limit of detection.- RSD = Relative standard deviation
S. D. = Standard deviation

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