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Development and Validation of Absorbance ratio method for Simultaneous Determination of Cefpodoxime Proxetil and Ofloxacin in combined tablet dosage form.

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ABSTRACT

The present manuscript describe simple, sensitive, rapid, accurate, precise and economical Q-absorbance ratio method for the simultaneous determination of Cefpodoxime proxetil and ofloxacin in combined tablet dosage form. Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the λ-max of one of the two components. Cefpodoxime proxetil and ofloxacin show an isoabsorptive point at 272 nm in methanol. The second wavelength used is 236 nm, which is the λ-max of Cefpodoxime proxetil in methanol. The linearity was obtained in the concentration range of 5-17 µg/ml for both Cefpodoxime proxetil and Ofloxacin. The concentrations of the drugs were determined by using ratio of absorbances at isoabsorptive point and at the λ-max of ofloxacin. The method was successfully applied to pharmaceutical dosage form because no interference from the tablet excipients was found. The results of analysis have been validated statistically and by recovery studies.

Key Words: Cefpodoxime proxetil, Ofloxacin, Absorption ratio method Tablets, Validation.

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INTRODUCTION:

Cefpodoxime proxetil (CPD) (Figure 1) is chemically, 1-(isopropoxy carbonyloxy) ethyl (6R, 7R)-7-[2-(2-amino-4-thiazolyl)-(z)-2-(methoxyimino) acetamido]-3-methoxymethyl-3-cephem-4-carboxylate [1,] is a third generation cephalosporin antibiotic. It is used for infections of the respiratory tract, urinary tract and skin and soft tissues [2]. Cefpodoxime proxetil is official in IP and USP. IP [3] and USP [4] describe liquid chromatography method for its estimation. Literature survey reveals HPTLC [5] method for the determination of CPD. Literature survey also reveals RPHPLC [6] and spectrophotometric [7] methods for determination of CPD with other drugs. Ofloxacin (OFL) (Figure 2) is chemically, 9-fluro-2-3 dihydro-3-methyl-10-(4-methyl 1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]1,4benzoxazine-6-carboxylic acid [8], is a fluoroquinolone antibacterial agent used in the treatment of chalmydia or chlamydophila infections including nongonococcal urethritis and in mycobacterial infections such as leprosy.[9.] It is official in IP, BP and USP. IP [10], BP [11] and USP [4] describe potentiometric method for its estimation. Literature survey reveals first derivative fluorescence spectroscopy [12], HPLC with fluorescence detector for estimation of Ofloxacin in human plasma [13]. Literature survey also reveals spectrophotometric [14], RP-HPLC and HPTLC [15] methods for determination of OFL with other drugs. The combined dosage forms of CPD and OFL are available in the market and used in Urinary tract infection and Typhoid. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of CPD and OFL in their combined dosage forms. Literature survey does not reveal any simple spectrophotometric or chromatographic method for simultaneous estimation of CPD and OFL in combined dosage forms. The present communication describes simple, sensitive, rapid, accurate, precise and economical spectrophotometric method based on simultaneous equation for estimation of both drugs in their combined tablet dosage forms.

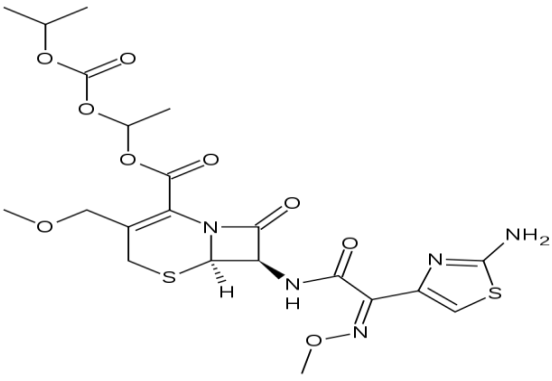

Figure 1: **Chemical structure of CPD**

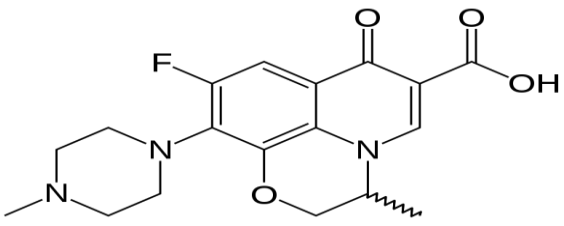

Figure 2: Chemical structure Of OFL

MATERIALS AND METHODS

Apparatus

A shimadzu model 1700(Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software (UV Probe version 2.31). A Electronic analytical balance (Acculab), and an ultrasonic bath was used in the study.

Reagents and Materials

CPD and OFL bulk powder was gifted by Corona Remedies Pvt. Ltd., Ahmedabad, and Gujarat, India. The commercial fixed dose combination product was procured from the local market. Methanol AR Grade was procured from S.D.Fine Chemicals Ltd., Mumbai, India.

Preparation of standard stock solution

An accurately weighed quantity of CPD (10 mg) and OFL (10 mg) were transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with methanol to obtain standard solution having concentration of CPD (100 µg/ml) and OFL (100 µg/ml).

Method

Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the λ-max of one of the two components. From the overlay spectra of two drugs, it is evident that CPD and OFL show an isoabsorptive point at 272 nm. The second wavelength used is 236 nm, which is the λ-max of CPD. Seven working standard solutions having concentration 5, 7, 9, 11, 13, 115 and 17 µg/ml for CPD and OFL were prepared in methanol and the absorbances at 272 nm (isoabsorptive point) and 236 nm (λ-max of CPD) were measured and absorptivity coefficients were calculated using calibration curve. The concentration of two drugs in the mixture can be calculated using following equations.

$$CX = [(QM - QY) / (QX - QY)] \times A1/aX1 \quad (1)$$

$$CY = (A1/aX1) - CX \quad (2)$$

Where, A1 and A2 are absorbances of mixture at 272 nm and 236 nm; aX1 and aY1 are absorptivities of CPD and OFL at 272 nm; aX2 and aY2 are absorptivities of CEF and OFL respectively at 236 nm; QM = A2 / A1, QX = aX2 / aX1 and QY = aY2 / aY1.

Validation of proposed method

Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 5-17 µg/ml for each CPD and OFL. Accurately measured standard stock solutions of each CPD and OFL (0.5, 0.7, 0.9, 1.1, 1.3, 1.5 and 1.7 ml) were transferred to a series of 10 ml volumetric flask separately and diluted up to the mark with methanol. The absorbances of solution were then measured at 272 nm and 236 nm. The calibration curves were constructed by plotting absorbances versus concentration and the regression equations were calculated.

Precision

System Precision

Intraday

The precision was checked by repeated scanning and measurement of the absorbances of standard solutions (n = 6) of CPD and OFL (5 µg/ml for both drugs) without changing the parameters of the proposed method for 3 times in a day.

Interday

The precision was checked by repeated scanning and measurement of the absorbances of standard solutions (n = 6) of CPD and OFL (5 µg/ml for both drugs) without changing the parameters of the proposed method for 3 consecutive days.

Method Precision

Intraday

The precision was checked by repeated scanning and measurement of the absorbances of sample solutions (n = 6) of CPD and OFL (5 µg/ml for both drugs) without changing the parameters of the proposed method for 3 times in a day.

Interday

The precision was checked by repeated scanning and measurement of the absorbances of sample solutions (n = 6) of CPD and OFL (5 µg/ml for both drugs) without changing the parameters of the proposed method for 3 consecutive days.

Accuracy

Accuracy was determined by calculating recovery of CPD and OFL by the standard addition method. From working sample solution of test (100 µg/ml of both), 0.5 ml of solution were taken and increasing aliquots of combined working standard solution (0.4, 0.5, and 0.6 ml from 100 µg/ml of both) were added and diluted to 10 ml with methanol. These solutions were prepared in triplicate. Absorbance of solution was measured at selected wavelength for CPD and OFL. The amounts of CPD and OFL were estimated by applying obtained values to the respective regression line equations.

Limit of detection and Limit of quantitation

The limit of detection (LOD) and the limit of quantitation (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Specificity

Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix. Commonly used excipients in tablet preparation were spiked in a pre weight quantity of drug and then absorbance was measured and calculation done to determine quantity of drugs.(Figure 4)

Analysis of CPD and OFL in combined tablet

Twenty tablets were weighed and the average weight was calculated. The tablet powder equivalent to 10 mg of CPD and 10 mg of OFL were weighed and transferred to 100 ml volumetric flask. Methanol (50 ml) was added and sonicated for 20 min. The volume is adjusted up to the mark with methanol. The solution was then filtered through Whatman filter paper no. 41. The solution was suitably diluted with methanol to get a final concentration of 5 µg/ml of CPD and 5 µg/ml of OFL. The absorbances of the sample solution i.e. A1 and A2 were recorded at 272 nm (isoabsorptive point) and 236 nm (λ-max of CPD) respectively, and ratios of absorbance were calculated, i.e. A2/A1. Relative concentration of two drugs in the sample was calculated using above equation (1) and (2).

RESULT AND DISCUSSION

In absorbance ratio method (Q-analysis), the primary requirement for developing a method for analysis is that the entire spectra should follow the Beer's law at all the wavelength [16], which was fulfilled in case of both these drugs. The two wavelengths were used for the analysis of the drugs were 272 nm (isoabsorptive point) and 236 nm (λ-max of CPD) at which the calibration curves were prepared for both the drugs. The overlain UV absorption spectra of CPD (237 nm) and OFLO (299 nm) showing isoabsorptive point (272 nm) in methanol is shown in Figure 3. Regression Analysis Data and Summary of Validation Parameters are shown in Table 1. The validation parameters were studied at all the wavelengths for the proposed method. Accuracy was determined by calculating the recovery and the mean was determined (Table 2). The method

was successfully used to determine the amounts of CPD and OFL present in the tablet dosage forms. The results obtained were in good agreement with the corresponding labeled amount (Table 3). Precision was calculated as repeatability and intra and inter day variations (% RSD) for both the drugs. The Specificity spectra shows that no interference of placebo at 272 nm (isoabsorptive point) and 236 nm (λ-max of CPD) which is shown in Figure 4.

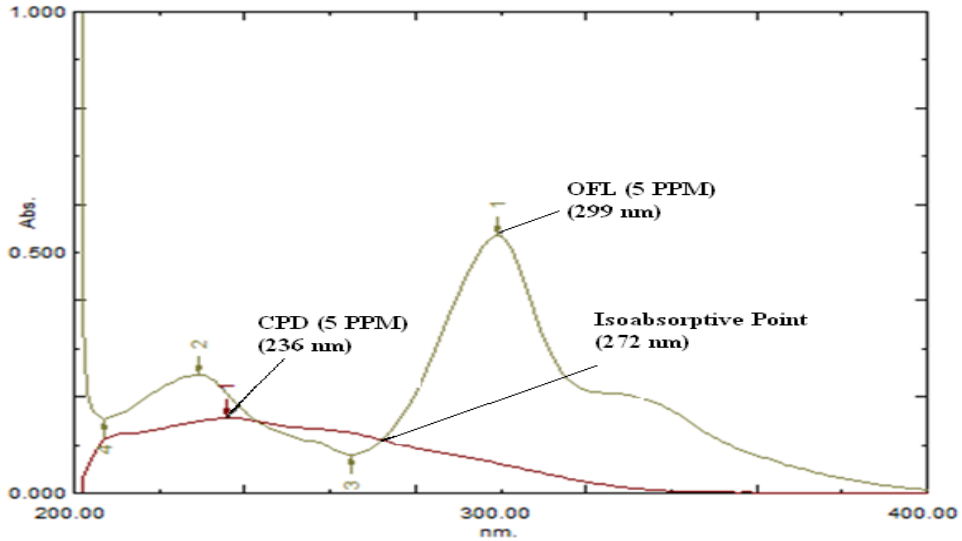

Figure 3:-Overlain absorption spectra of CPD (236 nm) and OFL (299 nm) showing isoabsorptive point (272 nm) in methanol.

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

Sr.No	Parameters	CPD	OFL	CPD and OFL
1	Wavelength range (nm)	236	236	272
2	Beer's law limit (µg/ml)	5-17	5-17	5-17
3	Regression equation (y = mx + c)	y = 0.0312x - 0.0006	y = 0.1024x + 0.0232	y =0.021x + 0.0002
4	Slope	0.0312	0.1024	0.021
5	Intercept	0.0006	0.0232	0.0002
6	Correlation Coefficient (r^2)	0.9999	0.9996	0.9999
7	System Precision (%R.S.D)[a]			
	1.Intraday Precision(n = 3)	0.77-1.100%	0.211-0.560%	0.88-1.20%
	2.Interday Precision(n = 3)	0.88-1.78 %	0.43-0.86 %	0.95-1.87%
8	Method Precision (%R.S.D)[a]			
	1.Intraday Precision(n = 3)	0.89-1.200%	0.235-0.652%	0.99-1.52%
	2.Interday Precision(n = 3)	0.89-1.320 %	0.252-0.720 %	0.98-1.452%
9	Accuracy (% recovery) (n = 3)	99.98-101.35%	98.11-101.44%	-
10	LOD[b] (µg/ml)	0.317	0.083	0.36
11	LOQ[c] (µg/ml)	0.961	0.253	0.952
12	Specificity	Specific	Specific	Specific
13	Assay (±S.D.)[d](n = 3)	100.8 ± 0.84	97.6 ± 1.47	-

[a]RSD = Relative standard deviation. [b]LOD = Limit of detection. [c]LOQ = Limit of quantitation
[d]SD is Standard deviation and n is number of replicates.

Table 2: Recovery data of proposed method

Drug	Level	Amount taken (µg/ml)	Amount added (µg/ml)	Amount added (%)	% Mean recovery (±S.D.) (n = 3)
	I	5	4	80	100.49 ± 1.74
CPD	II	5	5	100	99.98 ± 1.18
	III	5	6	120	101.35 ± 1.36
	I	5	4	80	98.11 ± 0.43
OFL	II	5	5	100	98.17 ± 0.86
	III	5	6	120	101.44 ± 1.12

S.D is Standard deviation and n is number of replicates.

Table 3: Analysis of CPD and OFL by proposed method

Tablet	Labeled claim (mg)		Amount found (mg)		% Label claims (±S. D.) (n = 3)	
	CPD	OFL	CPD	OFL	CPD	OFL
I	200	200	201.6	195.2	100.8 ± 0.84	97.6 ± 1.47

S.D is Standard deviation and n is number of replicates.

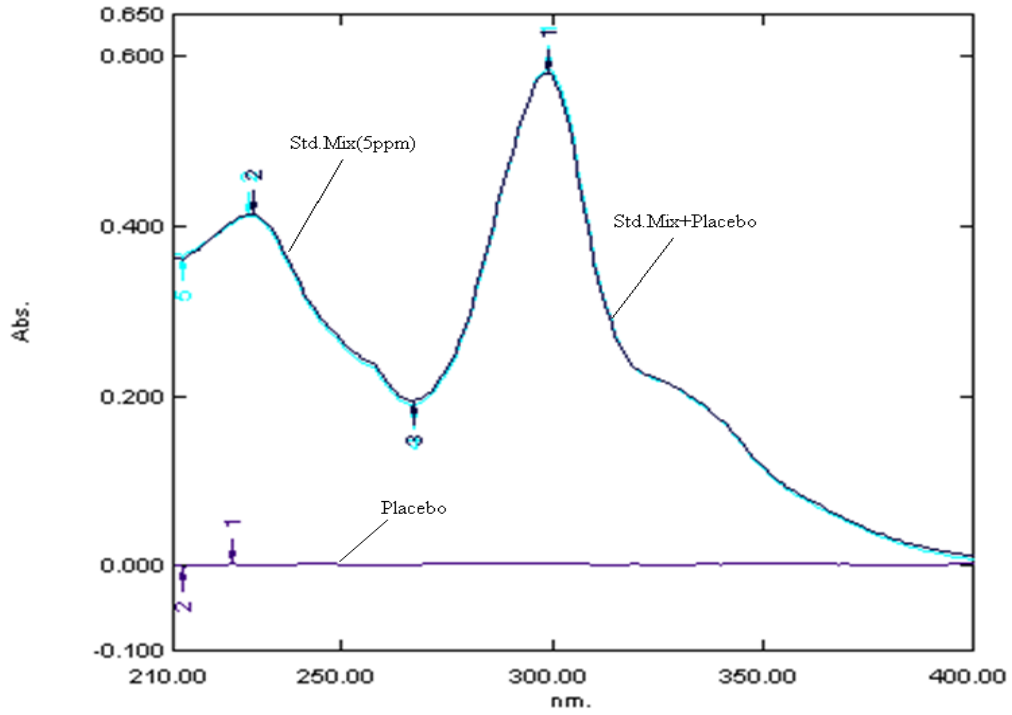

Figure 4: Overlain spectra of placebo, Standard Mixture & Placebo+Std.Mix. Mix

TABLES

CONCLUSION

The developed simultaneous equation method is found to be simple, sensitive, accurate and precise and can be used for routine analysis of CPD and OFL. The developed method was validated as par ICH guidelines. Statistical analysis proved that the method is repeatable and selective for the analysis of CPD and OFL in combination as a single drug in bulk as well as in pharmaceutical formulations.

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