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Research Article

VALIDATED SIMULTANEOUS UV SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF CIPROFLOXACIN AND TINIDAZOLE IN TABLET DOSAGE FORM

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ABSTRACT

The present study describes new, simple, rapid and novel spectrophotometric methods for simultaneous estimation of Ciprofloxacin (CPX) and Tinidazole (TZ) in tablet dosage form. For this, simultaneous Equation method (method A), Absorbance ratio method (Method B) and First order derivative spectroscopy (method C) are used. The method A involved measurement of absorbance at two wavelengths, 276 nm and 317 nm, λmax of CPX and TZ respectively. For method B, wavelengths 298 nm (isobestic point) and 276 nm (λmax of CPX) were used. For method C, wavelengths 262 nm (Zero crossing of CPX) and 307 nm (Zero crossing of TZ) were used for determination of TZ and CPX respectively. Beer's law was obeyed in concentration range of 2-10 µg/ ml and 2.4-12 µg/ ml for CPX and TZ respectively by all the methods.

The proposed methods are recommended for routine analysis in pharmaceutical formulations without interference of the excipients, since they are rapid, simple, accurate and also sensitive and specific. These methods were validated for linearity, accuracy and precision as per ICH guidelines.

Keywords: Ciprofloxacin, Tinidazole, Simultaneous, Spectrophotometry

INTRODUCTION

Ciprofloxacin Hydrochloride (1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid) is fluoroquinolone and antimicrobial with potent activity against a broad spectrum of bacteria. Tinidazole (1-(2-ethylsulfonylethyl)-2-methyl-5-nitro-imidazole) is antiprotozoal and antibacterial drug. These drugs are being used either alone or in combination for the treatment of diarrhea and dysentery of amoebic, bacterial or mixed origin. Literature survey revealed a few UV-Visible spectrophotometric[1-7] and HPLC[8-13] methods for the estimation of Ciprofloxacin Hydrochloride and Tinidazole alone or in combination with other drugs. Fixed dose combination containing CPX and TZ in tablet dosage form is recently available in the market and a single method using 2M urea as a solvent by simultaneous equation and absorbance ratio method has been reported for the simultaneous estimation of these drugs[14]. No method has been reported using Distilled water and by First order derivative spectroscopy for the simultaneous estimation of both these drugs.

MATERIALS AND METHODS

Instrumentation:

A Double beam UV-Visible spectrophotometer (Jasco V 530) with 10 mm matched quartz cells was used. All weighing were done on single pan balance (Shimadzu).

Reagents and chemicals

CPX and TZ reference standards were kindly provided by Litaka Pharmaceuticals Pvt. Ltd, Pune. All the reagents were of analytical grade. The Tablet Ciplox-TZ containing ciprofloxacin hydrochloride (500mg) and Tinidazole (600mg) was purchased from local market. CPX and TZ are available in the ratio of 5:6 respectively in the formulation and were used in same ratio for preparation of calibration curves.

Determination of absorptivity values

Standard stock solutions of CPX (100 µg/ml) and TZ (100 µg/ml) were prepared in Distilled water. For the selection of analytical wavelength solutions of CPX and TZ were prepared separately by appropriate dilution of standard stock solution with Distilled water and scanned in the spectrum mode from 200 to 400 nm. From the overlain spectra of these drugs [Figure 1], wavelengths 276 nm (λ_{max} of CPX), 317 nm (λ_{max} of TZ) and 298 nm (isobestic point) were selected for analysis. The calibration curves for CPX and TZ [Figure 2 and 3] were plotted in the concentration range of 2-10 µg/ml for both drugs at the selected wavelengths and absorptivity values were determined.

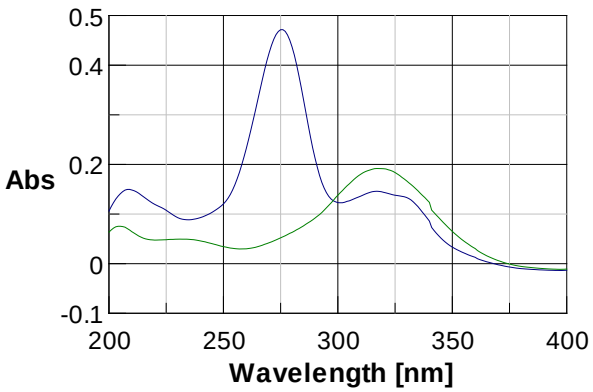

Fig. 1: It shows Overlain spectrum of CPX and TZ

Determination of Linearity

Standard stock solutions of pure drugs containing 25 mg of CPX and 30 mg of TZ/100 ml were prepared in distilled water. The working standard solutions were obtained by dilution of the stock solution in distilled water. Series of solutions with conc. 2-10 µg/ml and 2.4 – 12 µg/ml of CPX and TZ respectively were used to determine linearity by three methods. Solutions were scanned and Beers Lamberts law limit was determined.

Formulation analysis

For estimating CPX and TZ in marketed formulation, twenty tablets were weighed and finely powdered. A accurately weighed sample equivalent to CPX (25 mg) and TZ (30mg) was taken in a volumetric flask (100 ml), distilled water (50 ml) was added and sonicated for 15 min. Volume was made up to the mark with distilled water. The solution was then filtered though Whatmann filter paper No. 41. Appropriate aliquots were taken for further analysis.

Method A- Simultaneous Equation Method

Sample stock solution was appropriately diluted with distilled water to obtain final concentration of 8 µg/ml for CPX and 9.6µg/ml for TZ. Absorbance of diluted sample solution was measured at selected wavelengths. The concentration of drugs was determined by using the Equations 1 and 2. Using absorptivity values following equations

were developed for determining concentration of CPX and TZ in tablet formulation.

$A_1 = 82.58\ C_{CPX} + 5.8082\ C_{TZ}$ ---------- (1)

$A_2 = 26.075\ C_{CPX} + 35.442\ C_{TZ}$ ---------- (2)

Where, A_1 and A_2 are absorbances of the test sample at 276 and 317 nm, respectively.

C_{CPX} and C_{TZ} are the concentrations of CPX and TZ in gms/lit

Method B- Absorption Ratio Method (Q Method)

For Q method, 298 nm (isobestic point) and 276 nm (λmax of CPX) were selected as wavelengths of measurements. Concentrations of CPX and TZ were determined using following equations.

$C_{CPX} = (Qm - Q_{TZ}).\ A_1 / (Q_{CPX} - Q_{TZ}).\ A_{CPX1}$

$C_{TZ} = (Qm - Q_{CPX}).\ A_1 / (Q_{TZ} - Q_{CPX}).\ a_{TZ1}$

Where, $Qm = A_2 / A_1$

$Q_{CPX} = a_{CPX2} / a_{CPX1}$ and $Q_{TZ} = a_{TZ2} / a_{TZ1}$

A_2 and $A_{1=}$ Absorbance of Mixture at 276 nm and 298 nm respectively

a_{CPX1} a_{TZ1} = absorptivity of CPX (34.276) and TZ (24.055) at 298 nm

a_{CPX2} and a_{TZ2} = absorptivity of CPX (82.582) and TZ (5.8082) at 276 nm

Method C- First Order Derivative Spectroscopy

Standard solutions of both drugs (2-10 µg/ml) were scanned separately in the range of 200-400 nm. These spectrums were converted to first order derivative spectra (Figure 4) by using derivative mode with 21 data point. For this method, 262 nm and 307 nm were selected as wavelengths of measurements for TZ and CPX respectively. There was proportionate increase in amplitude at 262 nm and 307 nm for TZ and CPX respectively.

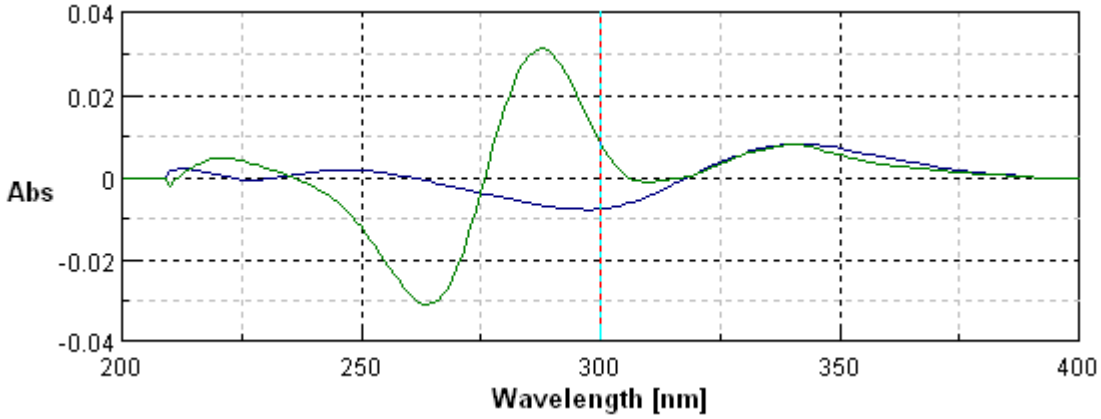

Fig. 4: It shows Overlain of Derivative Spectra of CPX and TZ

Analytical method validation[15, 16]

All the methods were validated for different parameters like linearity, specificity, accuracy and precision. Linearity was checked by calculating regression coefficient. The accuracy of the method was determined by calculating percentage drug recovery of CPX and TZ at three levels 50%, 100% and 150%. The Inter-day and intra-day precision of proposed method was determined. Drug concentrations of CPX and TZ mixture was prepared at three different times in a day and studied for intra-day variation. The same procedure was followed for six days in order to study inter-day variations. The percent relative standard deviation (% RSD) of prepared concentrations was analyzed for precision studies.

RESULTS AND DISCUSSION

The proposed methods for simultaneous estimation of CPX and TZ in combined dosage form were found to be accurate, simple and rapid which can be well understood from validation data as given in Table 1 to 4. The % R.S.D. was found to be less than 2, which indicates the validity of methods. Linearity was observed by linear regression equation method for CPX and TZ in different concentration range. The Correlation coefficient of these drugs was found to be close to 1.00, indicating good linearity in Table 1. The assay results obtained by proposed methods as shown in Table 2 are in fair agreement. Percentage drug recovery (± RSD) of CPX and TZ are shown in table 3. In all the cases RSD was not more than 2% depicting the accuracy of the developed method. Results of percentage drug recovery obtained from intra-day studies and inter-day studies are shown in table 4. From obtained data, it was found that RSD was not more than 2% indicating developed method has good repeatability. Hence it can be used for routine analysis of two drugs in combined dosage forms. These methods are accurate, simple, rapid, precise, reliable, sensitive, reproducible and economic and are validated as per ICH guidelines.

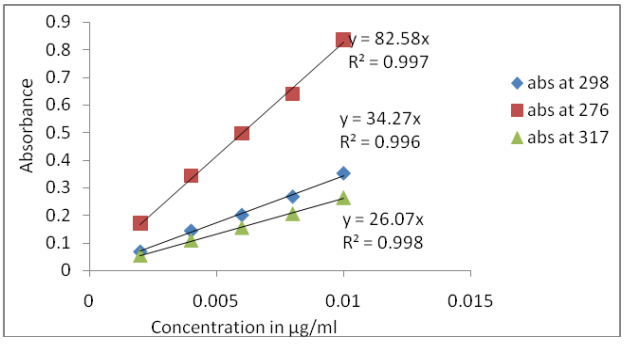

Fig. 2: It shows Calibration Curve for CPX

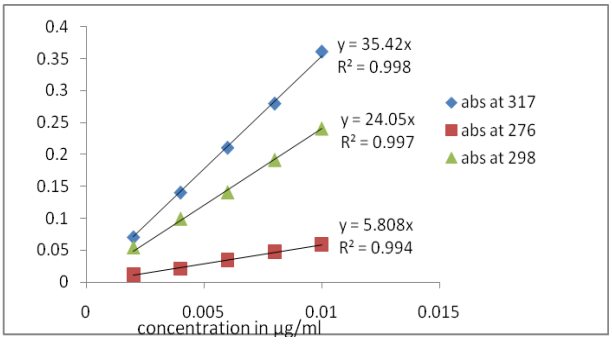

Fig 3: It shows Calibration Curve for TZ

Table 1: It shows Linearity data for CPX and TZ for all three methods

S. No.	Parameters	CPX			TZ		
		Method A	Method B	Method C	Method A	Method B	Method C
1	Linearity (µg /ml)	2-10	2-10	2-10	2.4-12	2.4-12	2.4-12
2	Correlation Coefficient (R^2)	0.9944	0.9971	0.9987	0.996	0.9985	0.9957

Table 2: It shows Assay results for tablet formulation by method A, B and C

Drug	Label Claim (µg/ml)	% Recovery ± S.D. Method A	% Recovery ± S.D. Method B	% Recovery ± S.D. Method C
CPX	8	99.52±1.014	98.78±0.402	101.14±0.5856
TZ	9.6	99.15±0.8117	98.98±0.828	99.25±0.7231

n=6

Table 3: It shows statistical analysis for Accuracy of the proposed methods

Drug	Concentration (µg/ml)		%Recovery			% R.S.D		
	Amount taken	Amount added	Method A	Method B	Method C	Method A	Method B	Method C
CPX								
50 %	4	2	99.16	98.75	100.50	0.9958	0.9905	1.01
100%	4	4	98.50	101.2	101.36	1.0274	1.0544	1.056
150%	4	6	100.78	98.52	99.89	0.6501	0.9348	1.051
TZ								
50 %	4.8	2.4	100.22	100.98	101.56	0.4612	0.4601	0.4598
100%	4.8	4.8	99.52	99.32	100.78	0.7867	0.7905	1.004
150%	4.8	7.2	100.5	101.45	101.67	1.0222	1.017	0.9982

n=3

Table 4: It shows statistical analysis for Precision of the proposed methods

I) Intraday Precision							
N=3 CPX				**TZ**			
Amt. of drug taken (µg/ml)	**% Recovery ± R.S.D**			**Amt. of drug taken(µg/ml)**	**% Recovery ± R.S.D**		
	A	**B**	**C**		**A**	**B**	**C**
8	100.86± 0.9932	98.46± 1.2011	99.53± 1.0023	9.6	99.56± 1.112	99.98± 1.009	101.46 ± 0.9821
II) Interday Precision							
N=6 CPX				**TZ**			
Amt. of drug taken (µg/ml)	**% Recovery ± R.S.D**			**Amt. of drug taken(µg/ml)**	**% Recovery ± R.S.D**		
	A	**B**	**C**		**A**	**B**	**C**
8	100.22± 0.9961	99.31± 1.010	99.83± 1.0003	9.6	100.34± 1.100	99.51± 1.003	100.72 ± 0.9853

CONCLUSION

Simple UV spectrophotometric methods were developed for the simultaneous determination of Ciprofloxacin and Tinidazole in Tablet formulation without any interference from the excipients. To the best of our knowledge, the present study is the first report for the purpose. The results of our study indicate that the proposed UV spectroscopic methods are simple, rapid, precise and accurate. Statistical analysis proves that, these methods are repeatable and selective for the analysis of CPX and TZ. It can therefore be concluded that use of these methods can save much time and money and they can be with accuracy.

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