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FIRST DERIVATIVE UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF CIPROFLOXACIN HYDROCHLORIDE AND TINIDAZOLE FROM A COMBINED DOSAGE FORM

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ABSTRACT

Ciprofloxacin hydrochloride (CPH) in combination with Tinidazole (TZ) is used in treatment of susceptible infections. The first derivative spectrophotometric method for simultaneous estimation of CPH and TZ from two component tablet dosage form has been developed and validated. In the present investigation an attempt has been made to develop accurate, reproducible, rapid and cost-effective method for estimation of CPH and TZ. The wavelengths selected for estimation of CPH and TZ are 263 nm and 304 nm, respectively. The beer's law was obeyed in the concentration range of 5-15µg/mL and 6-18µg/mL respectively for CPH and TZ. The proposed method was validated and successfully applied for the estimation of CPH and OZ in tablet formulations. The results suggested that the proposed method can be used for routine quality control of tablets containing CPH and TZ.

Key words: *Ciprofloxacin, Tinidazole, First derivative, spectrophotometric, Tablet.*

INTRODUCTION

The combination tablets of Ciprofloxacin hydrochloride and Tinidazole possess antibacterial and antidiarrheal agents which are prescribed for susceptible infections such as pulmonary infections, post operative anaerobic infections, appendicitis, diarrhea, dysentery, gynecological infections and respiratory tract infections. Ciprofloxacin is a broad spectrum antibiotic active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV enzymes necessary to separate bacterial DNA, thereby inhibiting cell division[1]. Chemically, Ciprofloxacin hydrochloride (CPH) is hydrochloride salt of 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxy-lic acid (Figure 1). Tinidazole (TZ) is chemically 1-(2-ethylsulfonylethyl)-2-methyl-5-nitro-imidazole (Figure 1) [2]. It is active against both protozoa and obligate anaerobic bacteria. It damages DNA strands or inhibit DNA synthesis in microorganism.

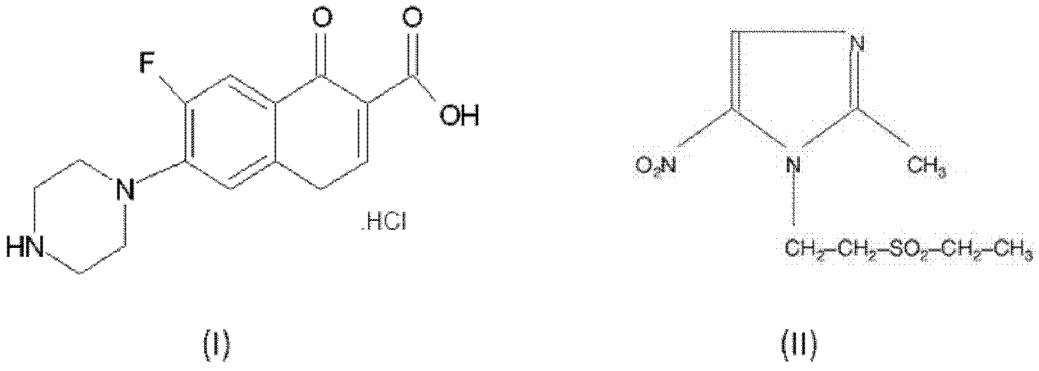

Figure 1: Chemical structure of (I) Ciprofloxacin hydrochloride and (II) Tinidazole

Literature survey revealed that various analytical methods such as spectrophotometric[3-5] , HPLC[6], differential pulse polarography[7] have been reported for the simultaneous estimation of both the drugs. The reported spectrophotometric methods are based on solving simultaneous equations and on the principle of Q-analysis. The aim of the present investigation is to develop a simple, sensitive, accurate and reproducible first order derivative UV Spectrophotometric method for the analysis of CPH and TZ in a combined tablet dosage form. Hence an economical method was developed and validated according to the ICH guidelines.

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EXPERIMENTAL

Instrumentation

Shimadzu UV/Visible double beam spectrophotometer (UV 1800) with 1 cm matched quartz cells was used for the spectral measurement. The

spectrophotometer was equipped with UV Probe software (2.35 ver).

Chemicals and Reagents

Acetic acid (AR grade) was purchased from Sisco Chem Pvt. Ltd, Andheri, Mumbai. Ciprofloxacin hydrochloride (99.47 %) and Tinidazole (98.29 %) were procured from Shalman Pharmaceuticals, Baroda as the gift samples. The commercial fixed dose combination of CPH and TZ was procured from local market.

Preparation of Stock Solutions

Accurately weighed 100 mg of CPH was transferred into 100 mL volumetric flask and dissolved in 0.1N acetic acid and diluted up to the mark with 0.1N acetic acid to get a stock solution containing 1mg/mL of CPH. Accurately weighed 100 mg of TZ was transferred into 100 mL volumetric flask and dissolved in 0.1N acetic acid and diluted up to the mark with 0.1N acetic acid to get a stock solution containing 1mg/mL of TZ.

Preparation of Working Standard Solutions

10 mL of CPH standard stock solution was diluted to 100 mL with 0.1N acetic acid to get CPH working standard solution containing 100 µg/mL of CPH. 10 mL of TZ standard stock solution was diluted to 100 mL with 0.1N acetic acid to get TZ working standard solution containing 100 µg/mL of TZ.**Selection of Analytical Wavelengths for CPH and TZ**

The standard solutions of 10µg/mL of CPH and TZ were scanned over 200-400 nm. Then ZCP was selected from the first derivative spectrum of CPH exhibits a maximum at 263 nm while TZ reads zero and TZ exhibits absorption at 304 nm while CPH reads zero. The concentration of CPH and TZ correlates very well with the measured first derivative peaks as established by quantitative investigations using regression analysis. Various ZCPs were obtained for CPH and TZ.

Calibration Curves for CPH and TZ

Different aliquots (2.50, 3.75, 5.00, 6.25 and 7.50 mL) were withdrawn from the working standard solution and transferred to 50 mL volumetric flasks and made up to mark with 0.1 N acetic acid to produce range of concentrations of 5 – 15 µg/mL of CPH.

Different aliquots (0.6, 0.9, 1.2, 1.5 and 1.8 mL) were withdrawn from the working standard solution and transferred to 10 mL volumetric flasks and made up to mark with 0.1 N acetic acid to produce range of concentrations of 6 – 18 µg/mL of TZ.

The first order derivative spectrum of each standard solution was recorded against acetic acid as a blank solution. The first derivative amplitudes were measured at wavelength of 263 nm for standard solutions of CPH and 304 nm for standard solutions of TZ. Calibration curves were constructed by plotting first derivative amplitude at the selected wavelengths against corresponding concentration of CPH and TZ. Regression equations for CPH and TZ were calculated from the corresponding calibration curves of CPH and TZ.

Validation of the Proposed Method

Linearity and Range

Different aliquots (2.5 mL, 3.75 mL, 5 mL, 6.25 mL and 7.5 mL) were withdrawn from standard stock solution containing 100 µg/mL of CPH and diluted up to 50 mL with 0.1N acetic acid to obtain linear concentration of CPH (5-15 µg/mL). Similarly aliquots of (0.6 mL, 0.9 mL, 1.2 mL, 1.5 mL and 1.8 mL) were withdrawn from working standard solution containing 100 µg/mL of TZ and diluted up to 10 mL with 0.1N acetic acid to obtain linear concentration of TZ (6-18 µg/mL). The solutions were analyzed and absorbance was measured and calibration curves were plotted against drug concentration.

Precision

Interday and Intraday precisions were evaluated by analyzing concentrations of CPH (5-15 µg/mL) and TZ (6-18 µg/mL) five times on the same day and on five different days. The % RSD was calculated to determine any intraday and Interday variation.

Repeatability

It was carried out using five replicates of standard mixture solution of CPH (10 µg/mL) and TZ (12 µg/mL).

Accuracy

Accuracy was determined by calculating the % recovery by standard addition method. Known amount of the standard solution of (0 µg/mL, 2.5µg/mL and 5µg/mL of CPH and TZ) were added in pre-analysed sample solution of marketed formulation (7.5µg/mL) which gave solution having strength of 75%, 100% and 125% of middle concentration from the range. Each solution was injected in triplicates and recovery was calculated by measuring the peak area and fitting themselves into the regression equation.

Limit of detection (LOD) and Limit of quantification (LOQ)

The limit of Detection (LOD) and limit of quantitation (LOQ) of the drugs were calculated by measuring the responses of the standard solutions below the range of calibration curve. The LOD and LOQ were calculated using equations as per ICH guideline i.e., LOD=3.3 (σ/S) and LOQ=10 (σ/S) where σ is standard deviation of the response and S is slope of the calibration curve.

Analysis of Marketed Formulation

Twenty tablets were accurately weighed and finely powdered. Accurately weighed tablet powder equivalent to 250 mg of CPH was transferred into 100 mL of volumetric flask, diluted up to the mark with 0.1N acetic acid and sonicated for 30 minutes. The resulting solution was filtered and 10 mL solution was diluted to 100 mL with 0.1N acetic acid. Further 4 mL aliquot was withdrawn and transferred to 100 mL volumetric flask to obtain the final solution containing 10µg/mL of CPH(correspondingly concentration of TZ would be 12 µg/mL). Sample solutions of CPH (10 µg/mL) and TZ (12 µg/mL) were analyzed three times. First derivative amplitudes of both the drugs were measured at the selected wavelengths and % assay was calculated using respective regression equations.

Results and Discussion

The zero order spectra of CPH and TZ showed

wavelength maxima at 276 nm and 316 nm, respectively (Figure 2). Overlapping of both the spectra led to

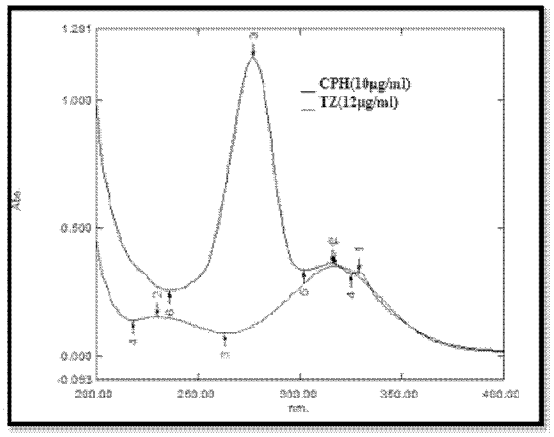

Figure 2: Overlain zero order spectra of CPH (10 µg/mL) and TZ (10 µg/mL)

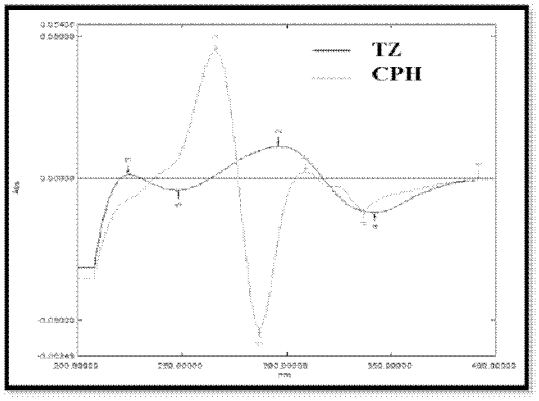

Figure 3: First Derivative Spectra of CPH (10 µg/mL) and TZ (12 µg/mL)

interference at respective wavelength maxima of the drugs. The first order derivatization of the normal spectra of CPH and TZ was carried out to resolve the overlapping spectra (Figure 3).

Selection of Wavelengths

The peaks, valleys and zero crossing points were observed for selection of wavelengths at which quantification of CPH and TZ can be done. The zero-crossing point of TZ where CPH showed maximum amplitude was found to be 263 nm and selected for analysis of CPH. The zero-crossing point of CPH where TZ showed maximum amplitude was found to be 304 nm and selected for analysis of TZ. The concentration of CPH and TZ correlates very well with the measured first derivative peaks as established by quantitative investigations using regression analysis.

Calibration curves for CPH and TZ

The overlain first order derivative UV spectra of CPH and TZ calibration standard solutions are shown in Figure 4 and 5 respectively. The representative calibration curves for CPH and TZ were constructed by plotting first derivative amplitudes at 263 nm and 304 nm against concentration range 5-15 µg/mL and 6-18µg/mL, respectively (n=5). The average linear regressed equations for the corresponding curves were y=0.00432x + 0.00067 (CPH) and y=0.0008x + 0.0004 (TZ) with co-relation coefficient values of 0.9958 and 0.9963.

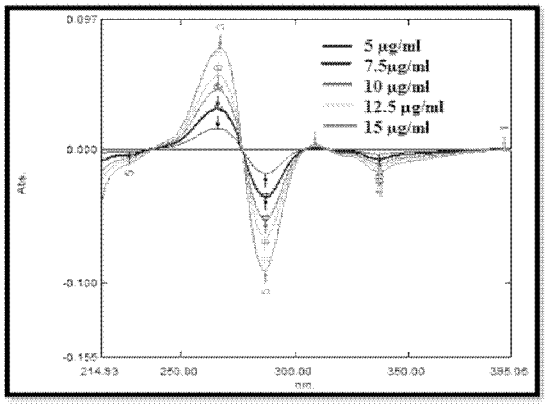

Figure 4: First Derivative Overlay Spectra of CPH (5-15 µg/mL)

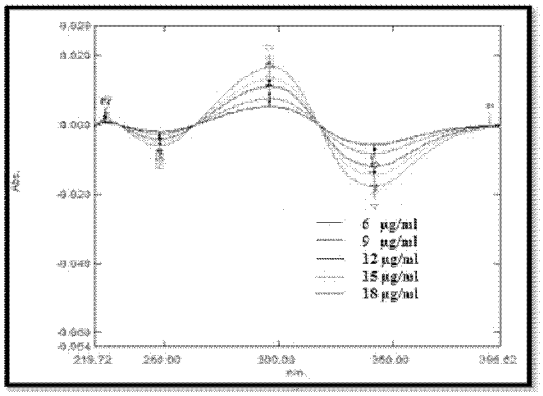

Figure 5: First derivative overlay spectra of TZ (6-18 µg/mL)

Method Validation

Linearity and Range

The linearity for CPH and TZ was found in the range of 5-15 µg/mL and 6-18 µg/mL, respectively. The linearity data for CPH and TZ are shown in Table 1 and 2 respectively.

Table 1: Linearity Data for CPH at 263 nm (n = 5)

Concentration of CPH (µg/mL)	First Order Amplitude for CPH Mean±SD	%RSD
5	0.023±0.0001	0.55
7.5	0.031±0.0001	0.32
10	0.043±0.0009	0.21
12.5	0.054±0.0002	0.43
15	0.066±0.0005	0.90

Table 2: Linearity Data for TZ at 304 nm (n = 5)

Concentration of TZ (µg/mL)	First Order Amplitude for TZ Mean±SD	%RSD
6	0.004±0.0006	1.35
9	0.006±0.0001	1.69
12	0.009±0.0004	0.48
15	0.012±0.0002	1.73
18	0.014±0.0009	0.68

Precision

The precision studies were performed at five different concentration levels over the linearity range. The interday and intraday precision data for CPH and TZ are respectively shown in Tables 3 and 4.

Table 3: Intraday and Interday Precision Data for CPH at 263 nm (n = 5)

Concentration of CPH (µg/ml)	Intraday Precision for CPH		Interday Precision for CPH	
	First Order Amplitude Mean±SD	%RSD	First Order Amplitude Mean±SD	%RSD
5	0.023±0.0001	0.55	0.023±0.0002	0.96
7.5	0.031±0.0001	0.72	0.032±0.0001	0.37
10	0.043±0.0019	1.41	0.043±0.0004	1.10
12.5	0.054±0.0009	1.03	0.053±0.0010	1.94
15	0.066±0.0005	0.90	0.065±0.0010	1.59

Table 4: Intraday and Interday Precision Data for TZ at 304 nm (n = 5)

Concentration of TZ (µg/mL)	Intraday Precision for TZ		Interday Precision for TZ	
	First Order Amplitude Mean±SD	%RSD	First Order Amplitude Mean±SD	%RSD
6	0.004±0.0006	1.93	0.004±0.006	1.35
9	0.006±0.0001	1.69	0.006±0.008	1.22
12	0.009±0.0002	0.48	0.009±0.001	1.18
15	0.012±0.0002	1.73	0.011±0.0001	1.14
18	0.014±0.0009	0.68	0.014±0.0003	0.20

Repeatability

The %RSD for measurement of first order amplitude for CPH and TZ were found to be 1.10 and 0.72, respectively.

Accuracy

The accuracy of the method was checked by spiking pre-analyzed solution with known amount of the drug and data obtained are shown in Table 5, which suggests that the recovery of the present method ranges from 98.4- 103.2 % for CPH and 95.2-102.7 % for TZ.

Table 5: Recovery Studies for CPH and TZ by Proposed Method (n=3)

Amount of Sample (µg/mL)		Amount of Standard Drug Added (µg/mL)		Amount Recovered		% Recovery ± SD	
CPH	TZ	CPH	TZ	CPH	TZ	CPH	TZ
7.5	9	0	0	7.38	8.57	98.4±0.09	95.2±0.58
7.5	9	2.5	3	10.32	12.32	103.2±0.12	102.7±0.57
7.5	9	5	6	12.59	14.95	100.6±0.11	99.7±0.65

LOD and LOQ

The LOD was found to be 0.196 µg/mL and 0.44 µg/mL for CPH and TZ respectively. The LOQ as calculated by standard formula was found to be 0.59 µg/mL and 1.25 µg/mL for CPH and TZ respectively.

Analysis of Marketed Formulations

Quantitative determination of CPH and TZ in tablets was performed using proposed method and results were in good agreement with labelled amount of CPH and TZ (Table 6).

Table 6: Analysis of Market Formulation (Tablets)

Market Formulation	Label Claim (mg/tablet)		Assay ± SD (% of Label Claim)	
	CPH	TZ	CPH	TZ
CIPLOX-TZ® Tablets	500	600	102.7±1.54	98.25±0.59

CONCLUSION

The proposed UV spectrometric method for quantitative estimation of CPH and TZ in combined dosage form is found to be simple, rapid, precise, and accurate. The excipients of the commercial sample analyzed did not interfere in the analysis, which proved the specificity of the method for this formulation. The developed method is found to be more reproducible and sensitive than other such reported method.

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REFERENCES

1. Drlica K, Zhao XK. DNA gyrase, topoisomerase IV, and the 4-quinolones. *Microbiology and Molecular Biology Reviews*, 61(3): 377-392, 1997.
2. *The Merck Index*. Whitehouse station NJ: Merck & Co., INC., 2006.
3. Bombale MV, Kadam SS, Dhaneshwar SR. Simultaneous spectrophotometric estimation of Ciprofloxacin and Tinidazole from a combined dosage form. *Indian Journal of Pharmaceutical Sciences*, 59(5):265-268, 1997.
4. Gummadi S, Thota D, Varri SV, Vaddi P, Jillella VLNSR. Development and validation of UV spectroscopic methods for simultaneous estimation of ciprofloxacin and Tinidazole in tablet formulation. International *Current Pharmaceutical Journal*, 1(10): 317-321, 2012.
5. Sharma R, Pathodiya G, Mishra PG, Sainy J. A novel spectrophotometric method for quantitative deter-mination of Ciprofloxacin hydrochloride and Tinidazole in tablets using hydrotropic solubilizing agent. *Journal of Pharmacy Research*, 4(3):859-861, 2011.
6. Bhatia MS, Kaskhedikar SG, Chaturvedi SC. High performance liquid chromatographic estimation of Ciprofloxacin hydrochloride and Tinidazole from tablets. *Indian Journal of Pharmaceutical Sciences*, 61(5):311-312, 1999.
7. Salvi VS, Sathe PA, Rege PV. Determination of Tinidazole and Ciprofloxacin Hydrochloride in Single Formulation Tablet using Differential Pulse Polarography. *Journal of Analytical & Bioanalytical Techniques*, 1:110. doi:10.4172/2155-9872.1000110, 2010