Quantitative Estimation of Ciprofloxacin and Tinidazole of Marketed Preparation using Hydrotropic agent

Suman Gehlot[1*], Debadash Panigrahi[2] and Raghvendra Dubey[1]
[1] COP, Dr. APJ Abdul Kalam University, Indore, (M.P.)
[2] Ujjain Institute of Pharmaceutical Sciences, Ujjain, (M.P.)
E.mail: sumangehlot81@gmail.com
Mob. 8120271389

ABSTRACT - The proposed method is new, simple, environmentally friendly, accurate, reproducible, precise and validated statistically for quantitative estimation ciprofloxacin and tinidazole in tablet dosage form The % of drug content by absorption ratio method of CFC and TIN in tablet dosage form was found to be with in the range of 99.70-100.30 % and 99.75-100.50% respectively. The result of recovery study shows that the % of recovery for both the drugs were with in the range of 99 – 100%. Precision study shows that the drug content for both drug in market formulation were within the range of 97 - 101%. In derivative spectroscopy method, the % of drug content in tablet dosage form was within the range of 99.98-100.20% and 99.86-100.08% for CFC and TIN respectively. The % of drug content of both drugs in recovery and precision study for tablet dosage form were with in the range of 98 -102%and 98-101% respectively.

Key Word: Ciprofloxacine, Tinidazole, Urea

INTRODUCTION

The term hydrotropy (mixed solvency) has been used to designate the increase in solubility of various substances in water due to the presence of large amounts of additives[1,2]. Chemically Ciprofloxacin Hydrochloride (CPH) is (1-cyclopropyl-6-fluoro-1, 4- dihydro-4-oxo-7-(1-piperazinyl)-3- quinolinecarboxylic acid) is fluoro quinolones and antimicrobials with potent activity against a broad spectrum of bacteria[3,4,5] Chemically Tinidazole (TZ) is (1-(2- ethylsulfonylethyl)-2-methyl-5-nitro-imidazo-le), antiprotozoal and anti-bacterial drugs[1,2] . These drugs are being used either alone or in combination for the treatment of diarrhoea and dysentery of amoebic, bacterial or mixed origin. Literature survey revealed that chromatographic method was reported for its estimation from tablet dosage form and spectrophotometric methods for estimation in combine dosage forms. But so far no spectrophotometric methods has been reported for quantitative estimation of CPH and TZ in combined dosage form, hence an attempt has been made to develop simple, sensitive, economical, rapid, precise and accurate methods to analyze the drugs simultaneously.

MATERIAL AND METHODS[6-9]

Protocol

Product Name : Bycep-TZ (Ciprofloxacin 500mg, Tinidazole 600mg)

Average weight : 1.31mg

Preparation of 0.1 M Urea Solution

6.01gm of Urea dissolve in small quantity of distilled water and made up to the volume 1000ml using distilled water.

Preparation of stock solutions

The standard stock solution of CFC and TIN were prepared by dissolving of 100mg of each drug in 100ml of 0.1M Urea Solution. Stock solution of CFC and TIN were further diluted in 0.1M Urea Solution to get solution working standard solution of concentration 100µg/ml.

Selection of appropriate wavelength

From standard stock solutions of 100µg/ml each of CFC and TIN dilutions of 50µg/ml were prepared for each drug respectively. Both the solutions were scanned over the range of 400-200nm in the spectrum mode maintained at slow scan speed and the overlain spectra of the two drugs were obtained. The overlain spectra exhibit absorbance maxima at 270nm and 319nm for CFC and TIN respectively and the isobestic point was found to be 293nm.

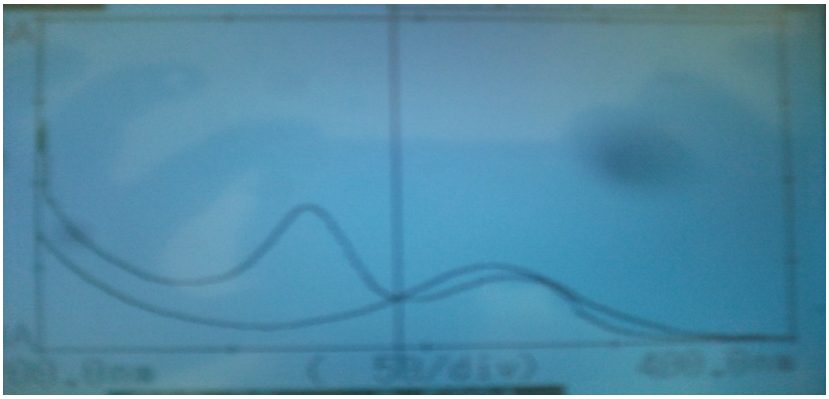

Fig.1 Overlain Spectra of CFC and TZ

Absorption ratio method

In absorption ratio method, absorbance of both the drugs were calculated at two selected wavelengths; among which λ_1 is the λmax of either drug among the drugs to be analyse and λ_2 is the wavelength of iso-bestic point (where both drugs show same absorbance). From the UV wavelength 293 (λ_2-isobestic point) and 270 (λ_1-λmax of CFC) were selected for study. The concentration of the individual drug components were calculated by using the following equations,

$$C_x = \frac{Q_m - Q_y}{Q_x - Q_y} \times \frac{A_1}{ax_1} \quad \text{.....Equation.1} \qquad C_y = \frac{Q_m - Q_x}{Q_y - Q_x} \times \frac{A_1}{ax_1} \quad \text{.....Equation. 2}$$

where $Q_m = \frac{A_2}{A_1}$, A_2 is absorbance of sample at λ_2 (isobestic point), A_1 is absorbance of sample at λ1 (λmax of CFC), $Q_x = \frac{ax_2}{ax_1}$, $Q_y = \frac{ay_2}{ay_1}$, ax_1, ax_2, ay_1 and ay_2 are the absorptivities values of CFC and TZ respectively.

Preparation of standards solutions for linearity study

Form the standard stock solutions of 100µg/ml of both CFC and TIN, different dilutions were prepared for each drug having concentration as shown in Table 1 and Table 2 with 0.1M Urea solution . Then these solutions were scanned over the range of 400-200nm and absorbance were measured in the spectrum mode at the respective analytical wavelengths 270 and 293nm for CFC and TIN respectively for all the five replicates. The calibration curves were plotted between the mean value of the observed absorbance and respective concentration. From the calibration curve, it was found that both the drug follows Beer's-Lamberts law within the range of 5-50µg/ml.

Table-1 Linearity of CFC at λ_{max} 270nm

Replicate no.	Concentration									
	5	**10**	**15**	**20**	**25**	**30**	**35**	**40**	**45**	**50**
Replicate no.1	0.384	0.557	0.719	0.900	1.409	1.212	1.409	1.584	1.784	1.950
Replicate no.2	0.385	0.554	0.718	1.100	1.410	1.213	1.410	1.585	1.785	1.951
Replicate no.3	0.386	0.556	0.720	0.900	1.411	1.214	1.411	1.586	1.786	1.952
Replicate no.4	0.383	0.554	0.721	0.800	1.412	1.215	1.412	1.587	1.787	1.953
Replicate no.5	0.383	0.552	0.717	1.100	1.413	1.216	1.413	1.588	1.788	1.954
Mean	0.384	0.554	0.719	0.900	1.043	1.214	1.411	1.586	1.786	1.952

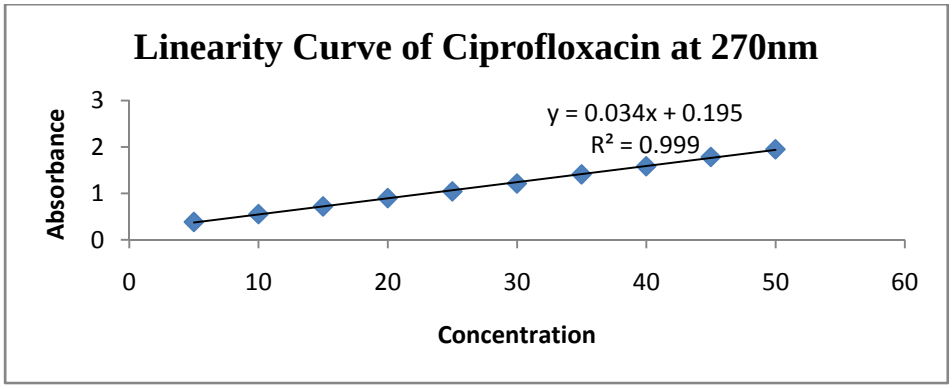

Fig. 2 Linearity of CFC at λ_{max} 270nm

Table-2 Linearity of TIN at λ_{max} 293nm

Replicate no.	Concentration									
	5	10	15	20	25	30	35	40	45	50
Replicate no.1	0.116	0.194	0.260	0.324	0.392	0.462	0.539	0.59	0.675	0.745
Replicate no.2	0.117	0.195	0.261	0.325	0.393	0.463	0.540	0.60	0.676	0.746
Replicate no.3	0.118	0.196	0.262	0.326	0.394	0.464	0.541	0.61	0.677	0.747
Replicate no.4	0.119	0.197	0.263	0.327	0.395	0.465	0.542	0.62	0.678	0.748
Replicate no.5	0.120	0.198	0.264	0.328	0.396	0.466	0.543	0.63	0.679	0.749
Mean	0.118	0.196	0.262	0.326	0.394	0.464	0.541	0.61	0.677	0.747

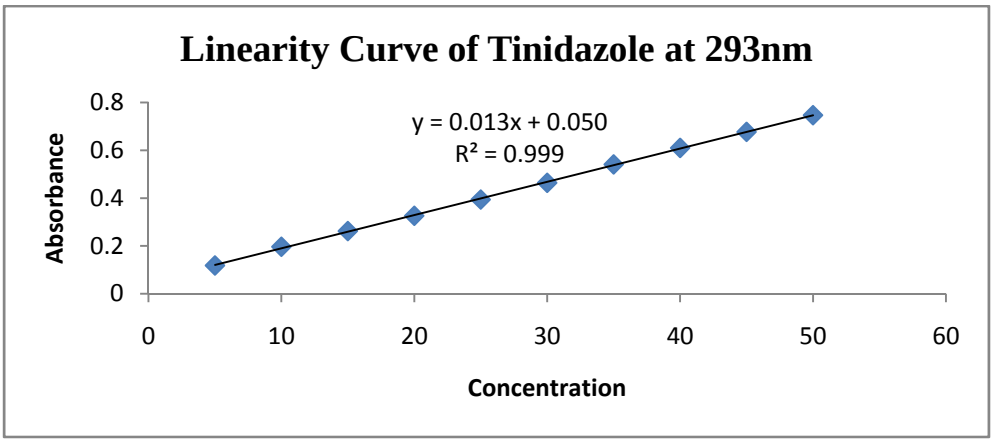

Fig. 3 Linearity of TIN at λ_{max} 293nm

Preparation of mixed standards

From the standard stock solutions of 100 µg/ml of the drugs different mixed standard solutions of known concentration were prepared as mention in the Table5.3. Then absorbance of these mixed standards were analyzed at the respective analytical wavelengths i.e 270 and 293nm respectively and percentage of drug content were calculated by applying these values in Eqn. 1 and 2.The results were reported in Table 3

Table-3 Analysis of mixed standard (Absorbtion Ratio Method)

Replicate no.	Lable claim (mg/Tab)		Conc. Found (mg/Tab)		Percentage found	
	CFC	TIN	CFC	TIN	CFC	TIN
Replicate-1	500	600	499.12	600.15	99.98	99.97
Replicate-2	500	600	500.01	600.22	100.03	100.02
Replicate-3	500	600	500.08	599.47	100.15	100.52
Replicate-4	500	600	500.32	600.08	100.02	99.88
Replicate-5	500	600	500.55	600.22	100.32	99.74

Analysis of Bycep-TZ Tablet

Ten tablets of Bycep-TZ were taken and their average weight was determined, they were crushed to fine powder. Then required quantity of powder was taken and dissolved in 50 ml of 0.1M Urea solution and adjust the volume upto 100 ml with 0.1M Urea solution to prepare a stock solution contain 1000 µg/ ml of CFC and 1300 µg/ ml of TIN. From the above prepared stock solution further sub stock solution of 130 µg/ml of CFC and 160 µg/ml of TIN were prepared. From the sub stock solution 1.3 ml was taken and dissolved up to 10 ml with 0.1M Urea solution to prepare a solution contain 25µg/ml of CFC and 32µg/ml of TIN. Absorbance of the prepared dilutions were determined at 270 and 293nm wavelengths in five replicates and their concentrations were determined by using the above simultaneous equations for CFC and TIN respectively. The result was given in the Table.4

Table-4 Result of Analysis of Bycep-TZ

Replicate no.	**Lable claim (mg/Tab)**		**Conc. Found (mg/Tab)**		**Percentage found**	
	CFC	**TIN**	CFC	**TIN**	CFC	**TIN**
Replicate-1	500	600	500.126	600.035	99.96	100.09
Replicate-2	500	600	500.016	600.012	100.06	100.02
Replicate-3	500	600	500.085	600.047	100.08	100.03
Replicate-4	500	600	500.132	600.058	100.02	99.81
Replicate-5	500	600	500.023	600.022	100.05	99.85

Recovery Study

Recovery study was carried out as per ICH guidelines, where to a pre analyzed solution of tablet formulation known concentration of standard solution was added equivalent to 80,100 and 120 of total drug content and the % of recovery was calculated. The results and its statistical validation values are given in Table 5.

Table-5 Result of Recovery Study

Replicate no.	**Amount taken (µg/ml)**		**Amount added µg/ml**			**% Recovery**	
	CFC	**TIN**	**%**	CFC	**TIN**	CFC	**TIN**
Replicate-1	15	19.5	80	12	15.6	100.01	98.96
Replicate-2	15	19.5		12	15.6	100.25	100.42
Replicate-3	15	19.5		12	15.6	99.86	100.00
Replicate-1	15	19.5	100	15	19.5	99.74	100.14
Replicate-2	15	19.5		15	19.5	99.86	100.03
Replicate-3	15	19.5		15	19.5	99.98	100.09
Replicate-1	15	19.5	120	18	23.4	100.11	101.01
Replicate-2	15	19.5		18	23.4	100.22	101.09
Replicate-3	15	19.5		18	23.4	100.05	100.01

Intermediate precision (inter-day and intra-day precision)

The intra and inter-day precision was calculated by assay of the sample solution on the same day and on different days at different time intervals respectively. To determine the intraday precision absorption of the prepared solutions of the tablet were taken on the very day at an interval of 1hr, 2hr and 3hr at the selected analytical wavelengths 270nm and 293nm respectively

Table -6 Intra and Inter day precision study of CFC

Replicate no.	**Percentage obtained**			**Percentage obtained**		
	1st hr	**2nd hr**	**3rd hr**	**Day 1**	**Day 2**	**Day 3**
Replicate-1	100.85	100.64	100.29	99.92	99.80	98.26
Replicate-2	100.78	100.20	100.18	99.84	99.81	98.71
Replicate-3	100.28	100.23	100.14	99.70	99.64	98.25
Replicate-4	100.26	100.18	100.12	99.98	99.86	98.84
Replicate-5	100.11	100.08	100.01	99.95	99.80	97.91
Mean	100.4	100.2	100.01	99.87	99.78	98.59

Table-7 Intra and Inter day precision study of TIN

Replicate no.	Percentage obtained			Percentage obtained		
	1st hr	2nd hr	3rd hr	Day 1	Day 2	Day 3
Replicate-1	100.88	100.70	100.27	100.19	99.80	98.87
Replicate-2	100.28	100.20	100.04	100.00	99.84	98.80
Replicate-3	100.85	100.77	100.64	100.51	99.00	98.86
Replicate-4	101.04	100.00	99.89	99.70	99.25	99.00
Replicate-5	100.10	100.01	99.82	99.76	99.33	98.24
Mean	100.6	100.30	100.1	100.00	99.44	98.75

Derivative method

In this method 20µg/ml solution for both the drugs were prepared from the standard stock solution and scanned in the range of 400nm to 200nm. The spectra obtained were derivative in first order and then recorded, the overlain spectra CFC had zero crossing point at 268.5 nm while TIN had zero crossing point at 265nm. At the zero crossing point of CFC, TN showed a measurable dA/dλ where as at the zero crossing point of TIN, CFC showed appreciable dA/dλ. Hence both wavelengths 265 nm and 268.5 nm were selected as analytical wavelengths for estimation of CFC and TIN respectively.

Preparation of standards solutions for linearity study

Form the standard stock solutions of 100µg/ml of both CFC and TIN, different dilutions were prepared for each drug having concentration as shown in Table 8 and Table 9 with 0.1M Urea solution . Then these solutions were scanned over the range of 400-200nm and absorbances were measured in the spectrum mode at the respective analytical wavelengths 265 and 268.5nm for CFC and TIN respectively for all the five replicates. The calibration curves were plotted between the mean value of the observed absorbance and respective concentration. From the calibration curve given in Fig.4 and Fig.5, it was found that both the drug follows Beer's-Lamberts law within the range of 5-50µg/ml.

Table-8 Absorbance of CFC at 265nm

Replicate no.	5	10	15	20	25	30	35	40	45	50
Replicate no.1	0.0093	0.0133	0.0183	0.021	0.0271	0.0323	0.0374	0.039	0.0460	0.0512
Replicate no.2	0.9994	0.0134	0.0184	0.022	0.0272	0.0324	0.0375	0.040	0.0461	0.0513
Replicate no.3	0.0095	0.0135	0.0185	0.023	0.0273	0.0325	0.0376	0.041	0.0462	0.0514
Replicate no.4	0.0096	0.0136	0.0186	0.024	0.0274	0.0326	0.0377	0.042	0.0463	0.0515
Replicate no.5	0.0097	0.0137	0.0187	0.025	0.025	0.0327	0.0378	0.043	0.0464	0.0516
Mean	0.0095	0.0135	0.0185	0.023	0.0273	0.0325	0.0376	0.041	0.0462	0.0514

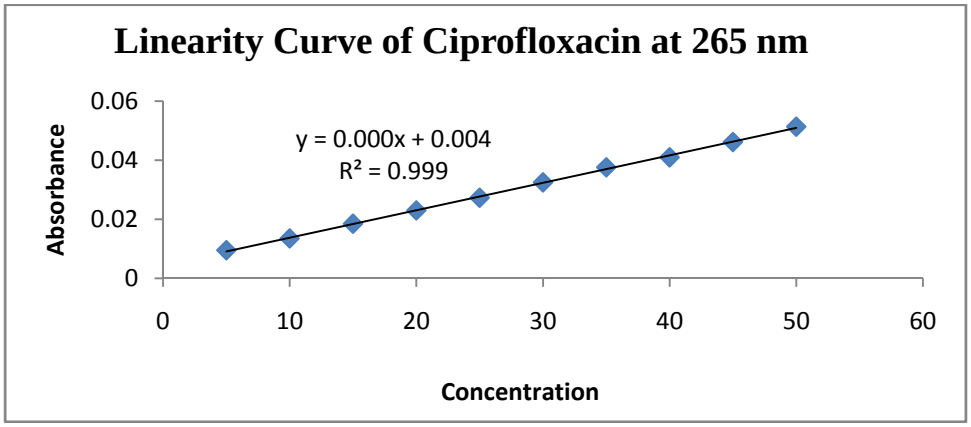

Fig. 4 Linearity Curve of CFC at 265nm

Table- 9 Absorbance of TIN at 268.5nm

Replicate no.	5	10	15	20	25	30	35	40	45	50
Replicate no.1	0.0063	0.0113	0.0160	0.0211	0.0270	0.0312	0.0377	0.0421	0.0470	0.0532
Replicate no.2	0.0064	0.0114	0.0161	0.0212	0.0271	0.0323	0.0378	0.0422	0.0471	0.0533
Replicate no.3	0.0065	0.0115	0.0162	0.0213	0.0272	0.0314	0.0379	0.0423	0.0472	0.0534
Replicate no.4	0.0066	0.0116	0.0163	0.0214	0.0273	0.0315	0.0380	0.0424	0.0473	0.0535
Replicate no.5	0.0067	0.0117	0.0164	0.0215	0.0274	0.0316	0.0381	0.045	0.0474	0.0536
Mean	0.0065	0.0115	0.0162	0.0213	0.0272	0.0314	0.0379	0.0423	0.0472	0.0534

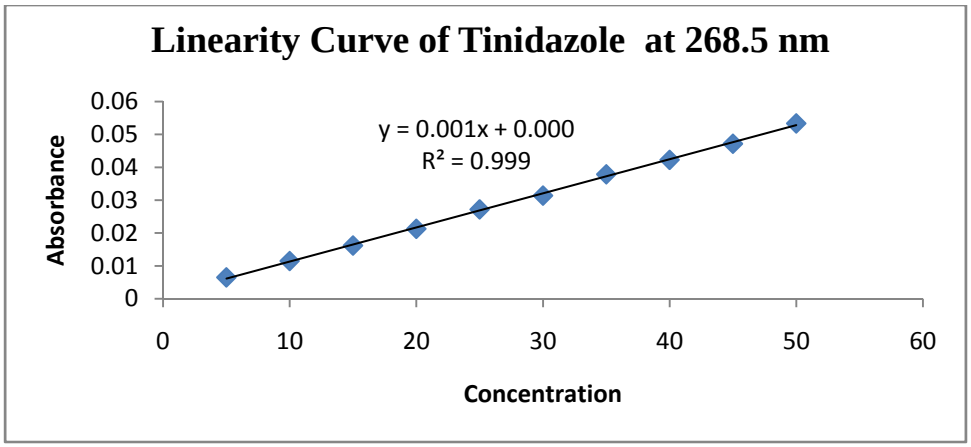

Fig. 5 Linearity Curve of TZ at 268.5nm

Preparation of mixed standards

Mixed standard analysis was performed to validate the procedure. From the standard stock solutions of 100 µg/ml of the drugs different mixed standard solutions of known concentration were prepared. Then absorbance of these mixed standards were analyzed at the respective analytical wavelengths i.e 265 and 268.5nm respectively and percentage of drug content were calculated. The results were reported in Table 10.

Table 10 Analysis of mixed standard

Replicate no.	Lable claim (mg/Tab)		Conc. Found (mg/Tab)		Percentage found	
	CFC	**TIN**	CFC	**TIN**	CFC	**TIN**
Replicate-1	500	600	500.036	600.023	100.27	100.06
Replicate-2	500	600	500.011	600.019	100.38	100.08
Replicate-3	500	600	500.128	600.019	100.05	99.83
Replicate-4	500	600	500.026	599.041	100.10	99.65
Replicate-5	500	600	500.0033	600.031	100.06	100.26

Analysis of Bycep-TZ

Ten tablets of Bycep-TZ were taken and their average weight was determined, they were crushed to fine powder. Then required quantity of powder was taken and dissolved in 50 ml of 0.1M Urea solution and adjust the volume upto 100 ml with 0.1M Urea solution to prepare a stock solution contain 1000 µg/ ml of CFC and 1300 µg/ ml of TIN. From the above prepared stock solution further sub stock solution of 130 µg/ml of CFC and 160 µg/ml of TIN. were prepared. From the sub stock solution 1.3 ml was taken and dissolved up to 10 ml with 0.1M Urea solution to prepare a solution contain 25µg/ml of CFC and 32µg/ml of TIN.. Absorbance of the prepared dilutions were determined at 265 and 268.5 nm wavelengths in five replicates and their concentrations were determined by using the above simultaneous equations for CFC and TIN respectively. The result was given in the Table 11.

Table-11 Result of Analysis of Byceptz

Replicate no.	Lable claim (mg/Tab)		Conc. Found (mg/Tab)		Percentage found	
	CFC	TIN	CFC	TIN	CFC	TIN
Replicate-1	500	600	500.036	600.023	100.27	100.06
Replicate-2	500	600	500.011	600.019	100.38	100.08
Replicate-3	500	600	500.128	600.019	100.05	99.83
Replicate-4	500	600	500.026	599.041	100.10	99.65
Replicate-5	500	600	500.0033	600.031	100.06	100.26

Recovery studies

Recovery study was carried out as per ICH guidelines, where to a pre analyzed solution of tablet formulation known concentration of standard solution was added equivalent to 80,100 and 120 of total drug content and the % of recovery was calculated. The results and its statistical validation values are given in Table 12.

Table-12 Result of Recovery Study

Replicate no.	Amount taken (µg/ml)		Amount added µg/ml			% Recovery	
	CFC	TIN	%	CFC	TIN	CFC	TIN
Replicate-1	15	19.5	80	12	15.6	99.66	100.26
Replicate-2	15	19.5		12	15.6	100.11	100.18
Replicate-3	15	19.5		12	15.6	100.32	100.03
Replicate-1	15	19.5	100	15	19.5	100.02	100.27
Replicate-2	15	19.5		15	19.5	98.94	101.03
Replicate-3	15	19.5		15	19.5	99.86	100.05
Replicate-1	15	19.5	120	18	23.4	100.21	100.20
Replicate-2	15	19.5		18	23.4	100.27	100.31
Replicate-3	15	19.5		18	23.4	99.87	100.06

Intermediate precision (inter-day and intra-day precision)

The intra and inter-day precision was calculated by assay of the sample solution on the same day and on different days at different time intervals respectively. To determine the intraday precision absorption of the prepared solutions of the tablet were taken on the very day at an interval of 1hr, 2hr and 3hr at the selected analytical wavelengths 265nm and 268.5nm respectively. Then the percentage of drug content were determined for both drugs. For determination of interday precision the same solutions were used to determine the absorbance at the particular analytical wavelengths on 1st, 2nd and 3rd day and calculate the percentage of drug content. The results of intra and inter day precision study for both drugs CFC and TIN are reported in Table 13 and 14.

Table-13 Intra and inter day precision study of CFC

Replicate no.	Percentage obtained			Percentage obtained		
	1st hr	2nd hr	3rd hr	Day 1	Day 2	Day 3
Replicate-1	100.85	100.60	100.29	100.11	99.00	98.15
Replicate-2	100.78	100.65	100.52	100.00	99.28	98.08
Replicate-3	100.25	100.21	100.02	99.81	99.70	99.80
Replicate-4	100.62	100.60	100.41	100.24	99.86	99.66
Replicate-5	100.92	100.80	100.70	99.98	99.82	98.62
Mean	100.6	100.5	100.3	100.02	99.44	98.86

Table-14 Intra and inter day precision study of TIN

Replicate no.	Percentage obtained			Percentage obtained		
	1st hr	2nd hr	3rd hr	Day 1	Day 2	Day 3
Replicate-1	100.21	100.15	100.11	100.12	99.32	98.85
Replicate-2	100.28	100.22	100.16	100.18	99.41	98.68
Replicate-3	100.58	100.43	100.32	99.93	99.28	98.52
Replicate-4	100.66	100.26	100.02	99.91	99.82	99.11
Replicate-5	100.45	100.20	99.97	99.63	99.02	98.94
Mean	100.43	100.2	100.11	99.95	99.37	98.82

RESULT AND DISCUSSION

Absorbtion ratio method

It is based on the fact of absorbed absorbance at any two seleceted wavelength for various concentration of a substance is constant.The proposed method for the simultaneous analysis of CFC and TIN use the absorbed absorbance at any two seleceted wavelenth. The two seleceted wavelength were 270nm (λ_{max} of CFC) & 293nm (isobestic point) by measuring the absorbance of the sample at 270nm & 293nm the amount of drug present is dosage form can be estimated . The percentage of drug content in the tablet dosage form was found to be 99.96 – 100.08% & 99.81 -100.09 % for CFC & TIN respectively. The method was validated as per ICH guidline. The validity & reliability of this proposed method was assessed by recovery study. The means of percent Recovery study were found to be <2.0 for both the the drugs, it relive that drug concentration could be accuratly determind by the prepared analyicatl method.

Derivative method

In this method, CFC had zero crossing point at 268.5 nm while TIN had zero crossing point at 265nm. At the zero crossing point of CFC, TIN showed a measurable dA/dλ where as at the zero crossing point of TIN, CFC showed appreciable dA/dλ. Hence both wavelengths 265 nm and 268.5 nm were selected as analytical wavelengths for estimation of CFC and TIN respectively. The percentage of dug content in tablet dosage form was estimated my determining the drug content in the solution of tablet at the above mentioned wavelengths. The percentage of drug content in the formulation was found to be 100.07 -100.38% for CFC and 99.65 – 100.26 % for TZ respectively. Recovery study was carried out as per ICH guideline were to a pre analysed solution of tablet, standard drug were added in 80,100 and 120 % of the % of drug content was determined. The % of drug recovery was with in the range of 98-102 % for both CFC and TZ.

Precision of the developed method was checked by performing intra day and inter day study. A pre analyzed tablet solution was analyzed for 3-conjugative hours and 3-conjugative days. The % of drug content of CFC and TZ were found to be 98 -101% both for intra day and inter day study.

REFERENCES

[1] Indian Pharmacopoeia, The Controller of Publication: New Delhi, Vol. 1, 1996: 764.
[2] British Pharmacopoeia, The Stationary Office: London, 1998:1296.
[3] United State Pharmacopoeia United State Pharmacopoieal Convention, 2003: 1263.
[4] Garcia, MS.; and Albero, MI.; Analysis of ciprofloxacin by High Performance Liquid Chromatography. Indian J Pharm Biopharm.; 2001; 61: 87.
[5] Sharma R:, Pathodiya G,; Mishra G.P.; Sainy J.; A Novel Spectrophotometric Methods for Quantitative Determination of Ciprofloxacin Hydrochloride and Tinidazole in Tablets using Hydrotropic Solubilizing Agent. Journal of Pharmacy Research 2011, 4(3), 859-861.
[6] Sharma R: Pathodiya G, Mishra G.P.; Qualitative and Quantitative estimation of water insoluble drugs from its formulations simultaneously: A Hydrotropic approach. S.J.Pharm.Sci.; 2010; 3(2): 37-42.
[7] Davidson, AG.; Beckett, AH.; Stenlake, JB.;Practical Pharmaceutical Chemistry, CBS Publishers & Distributor, 4th ed., New Delhi,2001.; 2: p. 286-288.
[8] Sharma R., Mishra G.P., Chaturvedi S. C.; Development and Validation of RP-HPLC Method for the Simultaneous Determination of Rabeprazole Sodium and Itopride Hydrochloride in Solid Dosage Form. EJournal of Chemistry, 2010, 7(3): 947-952.
[9] International Conference on Harmonization (ICH), Q2A: Text on Validation of Analytical Procedures: Definitions and Terminology, US FDA Federal Register, 60, (1995).