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Development and validation of stability indicating method for simultaneous estimation of ciprofloxacin hcl and tinidazole using rp-uplc method

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Abstract—This research manuscript describes simple, sensitive, accurate, precise and repeatable RP-UPLC method for the simultaneous determination of Ciprofloxacin HCl (CH) and Tinidazole (TZ) in tablet dosage form. The sample was analyzed by reverse phase C18 column (Purospher Star 100×2.1 mm, 2µm) as stationary phase and Phosphate Buffer: Acetonitrile (80:20) as a mobile phase and pH 3.0 was adjusted by ortho-phosporic acid at a flow rate of 0.3 ml/min. Quantification was achieved of Ciprofloxacin HCl at 278.5 nm and of Tinidazole at 317.5 nm with PDA detector. The retention time for Ciprofloxacin HCl and Tinidazole was found to be 1.71 and 2.22 minute respectively. The linearity for Ciprofloxacin HCl and Tinidazole was obtained in the concentration range of 3.125-43.75 µg/ml and 3.75-52.5 µg/ml with mean accuracies of 99.77% and 99.75% respectively. The method was successfully applied to pharmaceutical formulation because no chromatographic interferences from tablet excipients were found. The precision (intraday, interday and repeatability) of method was found within limits. The method was validated as per ICH guidelines. Ciprofloxacin HCl and Tinidazole API and market formulation were subjected to acid and alkali hydrolysis, oxidation, thermal and photolytic forced degradation. The degraded product peaks were well resolved from the pure drug peak with significant difference in their retention time values. Besides, the peak purity of drug substance and drug product peak also confirmed the specificity of the methods with respect to the degradation products. In the forced degradation study Ciprofloxacin HCl and Tinidazole showed maximum degradation in oxidation stress study followed by less degradation in acidic condition. The developed isogradient method was simple, specific, sensitive, and economic and can be used for estimation of Ciprofloxacin HCl and Tinidazole in bulk and their combined tablet dosage form for routine analysis and stability studies.

Keywords—Ciprofloxacin HCl, Tinidazole, Method validation, RP-UPLC, Forced degradation

I. INTRODUCTION

Ciprofloxacin HCl (CH), an antibacterial drug is widely used to treat a number of infections including: infections of bones and joints, endocarditis, gastroenteritis, malignant otitis externa, respiratory tract infections, cellulitis, urinary tract infections, prostatitis, anthrax, chancroid among others. Chemically it is the monohydrochloride monohydrate salt of 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid. And Tinidazole (TZ), an anti-parasitic drug chemically is 1-(2-ethylsulfonylethyl)-2-methyl-5-nitro-imidazole. Both drugs are official in Indian pharmacopeia, British Pharmacopeia and United States Pharmacopeia. The combination of CH and TZ is widely used in treatment of microbial infections. Literature search reveals that various analytical methods like UV-visible spectrophotometry (Rajesh Sharma et al.,2011); (Maliwal D et al.,2008); (Mashru R.C et al., 1998); Differential Pulse Polarography (Salvi V S et al., 2010); HPLC (M. S Bhatia et al.,1999); HPLC (Sani A. Ali et al.1,2011) have been reported for estimation of CH and TZ in their individual and combined dosage forms.. Literature survey describes that there is no reported method for degradation studies of Ciprofloxacin HCl and Tinidazole in various stress condition like alkaline, cidic, oxidative and thermal and photo degradation by RP-UPLC method. Therefore it was thought of interest to study the stability of Ciprofloxacin HCl and Tinidazole in various stress condition (alkaline, acidic, oxidative,

[1] CH-Ciprofloxacin HCl – CH, [2] Tinidazole- TZ
[3] RP-UPLC- Reverse Phase Ultra Performance Liquid Chromatography

Thermal and photo degradation) by RP-UPLC method. The chemical structure of Ciprofloxacin HCl and Tinidazole are shown in Fig.1 and Fig. 2.

II. MATERIALS & METHODS

2.1 Apparatus

The chromatography was performed on a Water (Acquity) RP-UPLC instrument equipped with PDA detector and Empower 2 software (Version- EMPOWER SOFTWARE 2 Build 2154); Purospher Star C18 column (100mm × 2.1 mm id, 2µm particle size, Merck, Germany) was used as stationary phase. Mettler Toledo analytical balance (Germany), pH meter from Lab India, an ultrasonic cleaner (Frontline FS 4, Mumbai, India), Hot air oven (Lab India), Photo stability chamber were used in the study.

2.2 Reagents and materials

Ciprofloxacin HCl and Tinidazole bulk powder was obtained from Nonlife Healthcare, division of Norma. Ahmedabad, India. Marketed Product (NIRCIP-TZ Tablet,
Batch No: 8F10042) was procured from the Nirlife Healthcare. Label claim of NIRCIP-TZ is Ciprofloxacin HCl -500 mg and Tinidazole-600 mg. Acetonitrile, KH2PO4 (Finar Reagent, Ahmedabad, India) used were of HPLC grade. Whatman filter paper no. 41. (Whatman International Ltd., England), Orthophosphoric acid(AR), Sodium Hydroxide, Hydrochloric acid, Hydrogen peroxide (30%) from Merck, specialties Pvt Ltd, Mumbai, India were used in the study.

2.3 Chromatographic Condition

Separation was achieved by using Purospher Star C18 column (100mm × 2.1 mm id, 2µm particle size, Marck, Germany) as stationary phase with Phosphate Buffer: Acetonitrile (80:20) as a mobile phase and P^H of 3.0 were adjusted by ortho-phosphoric acid at a flow rate of 0.3 ml/min and 6 min run time. Quantification was achieved of Ciprofloxacin HCl at 278.5 nm and of Tinidazole at 317.5 nm with PDA detector at 25± 2^oC temperature condition and 2µl injection volume.

2.4 Preparation of mobile phase

Accurately Weigh 6.8 gm of Potassium dihydrogen ortho phosphate was transferred into 1000 ml volumetric flask. Approximately 800 ml of water was added into the volumetric flask and sonicated. Volume was made up to 1000 ml with water. From this buffer solution 800 ml of solution was withdrawn and mixed with 200 ml of Acetonitrile into separated 1000 ml volumetric flask to make a mobile phase ratio buffer: Acetonitrile (80:20%v/v). P^H of 3.0 was adjusted by using ortho-phosphoric acid of mobile phase. This mobile phase used as diluents also throughout study.

2.5 Preparation of standard solution

2.5.1 Preparation of standard stock solution of Ciprofloxacin HCl (CH) (S1)

An accurately weighed 100 mg of quantity of CH reference standard was transferred into 100 ml volumetric flask, dissolved in 50 mL mobile phase and sonicated. After this it was diluted up to mark with mobile phase to get concentration of CH (1000µg/mL).

2.5.2 Working standard solution of CH (W1)

250 µg/ml of CH working standard solution was prepared by diluting 25 ml from above stock solution, made up to 100 ml with mobile phase into 100 ml volumetric flask**.**

2.5.3 Preparation of Tinidazole (TZ) stock solution (S2)

An accurately weighed 100 mg of quantity of TZ reference standard was transferred into 100 ml volumetric flask, dissolved in 50 mL mobile phase and sonicated. After this it was diluted up to mark with mobile phase to get concentration of TZ (1000µg/mL).

2.5.4 Working standard solution of TZ (W2)

300 µg/ml of TZ working standard solution was prepared by diluting 30 ml from above stock solution, made up to 100 ml with mobile phase into 100 ml volumetric flask**.**

2.6 Calibration curve of CH and TZ

2.6.1 Ciprofloxacin HCl

Aliquots of working standard solution (250µg/ml) of CH (1.25, 2.5, 5, 7.5, 10, 12.5, 15 and 17.5 ml) were transferred into a series of 100 ml volumetric flasks and volume was adjusted to the mark with mobile phase to get concentrations 3.125, 6.25, 12.5, 18.75, 25, 31.25, 37.50 and 43.75µg/ml. Solutions were injected into the system with stated chromatographic conditions..The graph of area of peak obtained versus respective concentration was plotted. The mean area and its standard deviation were calculated.

2.6.2 Tinidazole

Aliquots of working standard solution (300µg/ml) of TZ (1.25, 2.5, 5, 7.5, 10, 12.5, 15 and 17.5 ml) were transferred into a series of 100 ml volumetric flasks and volume was adjusted to the mark with mobile phase to get concentrations 3.75,7.5,15,22.5,30,37.5,45 and 52.5 µg/ml of TZ. Solutions were injected into the

system with stated chromatographic conditions. The graph of area of peak obtained versus respective concentration was plotted. The mean area and its standard deviation were calculated.

2.7 Preparation of Marketed sample solution for Assay

Simultaneous estimation of CH and TZ in marketed tablet dosage form (NIRCIP-TZ) containing label claim of CH-500 mg and TZ-600mg was carried out. In this assay procedure, 20 tablets of formulation were crushed and ground to a fine powder. Powder equivalent to 25 mg of CH and 30 mg of TZ was transferred to a 100 ml volumetric flask containing about 75 ml of mobile phase, dissolved and sonicated for 30 min. The solution was diluted up to the mark with mobile phase. The solution was filtered through Whatmann filter paper No. 41 and residue was washed with mobile phase. Accurately measured 1.0 ml of solution was transferred to 10 ml volumetric flask, diluted up to the mark with mobile phase to get final working concentration of CH (25 µg/ml) and TZ (30 µg/ml). A sample solution was injected under the operating chromatographic condition as described above and responses were recorded.

2.8 Method validation

The method was validated in compliance with ICH guidelines. (Q 2 B)

2.8.1 Linearity and Range

The linearity response was determined by analyzing 8 independent levels of calibration curve in the range of 3.125-43.75µg/ml and 3.75-52.5 µg/ml for CH and TZ respectively. Plot the calibration curve of Area versus respective concentration and find out correlation co-efficient and regression line equation for CH and TZ.

2.8.2 Precision

2.8.2.1 Repeatability

It was determined by analyzing CH (25µg/ml) and TZ (30µg/ml) seven times in mixture. The areas of seven replicate injections were measured and %RSD was calculated.

2.8.2.2 Intraday precision

For intraday, CH and TZ in the range of 3.125-43.75µg/ml and 3.75-52.5 µg/ml were analyzed three times on the same day and % C.V. was calculated.

2.8.2.3 Interday precision

For interday, AC and OM in the range of 3.125-43.75µg/ml and 3.75-52.5 µg/ml were analyzed on three different days and % C.V. was calculated.

2.8.3 Accuracy

The accuracy of the method was determined by calculating the recoveries of CH and TZ by the standard addition method. Known amounts of standard solutions of CH and TZ were at added at 80, 100 and 120 % level to prequantified sample solutions of CH and TZ (25 and30 µg/ml respectively). The amounts of CH and TZ were estimated by applying obtained values to the respective regression line equations, the solution was filtered through 0.45 µ Millipore PVDF filter; filtrate was collected after discarding first few ml. Each sample was prepared in triplicate at each level and injected. The chromatograms were recorded and from the peak area of drug, % recovery was calculated from regression equation of the calibration curve.

2.8.4 Limit of detection and Limit of quantification

The limit of detection (LOD) and limit of quantitation (LOQ) of the method were determined by standard deviation of response and slope method.

III. RESULTS AND DISCUSSION

To optimize the RP-UPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for Ciprofloxacin HCl and Tinidazole were obtained with a mobile phase comprising of Phosphate Buffer: Acetonitrile (80:20) and P^H of 3.0 adjusted by ortho-phosphoric acid at a flow rate of 0.3 ml/min to get better reproducibility and repeatability. Quantification was achieved of Ciprofloxacin HCl at 278.5 nm and of Tinidazole at 317.5 nm with PDA detector. The retention time for Ciprofloxacin HCl and Tinidazole were found to be 1.7 and 2.2 min, respectively. Linear correlation was obtained between peak area versus concentrations of Ciprofloxacin HCl and Tinidazole (Figure 3) in the concentration ranges of 3.125-43.75 µg/ml and 3.75-52.5 µg/ml with mean accuracies 99.77% and 99.75% The mean recoveries obtained were 99.77% and 99.75% for Ciprofloxacin HCl and Tinidazole, respectively, Table 1 and 2 which indicates accuracy of the proposed method. The % RSD values for CH and TZ were found to be <2 %, which indicates that the proposed method is repeatable. The low % RSD values of interday (0.08-0.46% and 0.22-0.4%) and intraday (0.08-0.62% % and 0.26-0.44 %%) variations for CH and TZ, respectively, reveal that the proposed method is precise. LOD values for Ciprofloxacin HCl and Tinidazole were found to be 0.063µg/ml and 0.01µg/ml respectively and LOQ values for Ciprofloxacin HCl and Tinidazole were found to be 0.193µg/ml and 0.030 µg/ml respectively (Table 1). The results of system suitability testing are given in (Table 3). The amount of Ciprofloxacin HCl and Tinidazole present in the marketed sample solutions were determined by plotting the

responses into the regression equations of the calibration curve for Ciprofloxacin HCl and Tinidazole, respectively and the results obtained were comparable with the corresponding labeled claim (Table 1).

3.1 Degradation study of CH and TZ in 0.1 N HCl at 70^{o}C for 4 hours in reflux condition.

It showed multiple peaks of degradation products. Major degradation peak was found at 0.6 min for CH and at 1.0 min for TZ in both drug product and drug substance. CH and TZ peak was observed at retention time 1.7 min and 2.3 min respectively. (Figure 4) The % drug degradation observed of CH and TZ was 11.98% and 8.37% respectively.

3.2 Degradation study of CH and TZ in 0.1 N NaOH at 70^{o}C for 4 hours in reflux condition.

It showed two peaks at retention time 0.9 min and 1.3 for CH in both drug product and drug substance. The degradation peak for TZ was found at 1.1 min in both drug substance and drug sample (Figure 5). The % drug degradation observed of CH and TZ was 17.54% and 27.72% respectively. This shows that Tinidazole is very prone to alkaline hydrolysis as compare to CH, and significant degradation of both the drugs was observed.

3.3 Oxidation degradation study of CH and TZ in 3 % H_2O_2 at 70^{o}C for about 1 hour in reflux condition.

Sample and drug substances were treated with 3 % solution of hydrogen peroxide and kept in water bath at 70^{o}C in reflux condition for about 1 hour. There was major degradation peak was found at 0.7 min for CH and TZ in drug substance and drug product. Another degraded peak for CH was found at 1.3 min and for TZ at 1.0 min. the % degradation observed of CH and TZ was 35% and 41.97% respectively. (Figure 6). From this it is observed that TZ showed maximum degradation in peroxide degradation condition.

3.4 Thermal Degradation study of CH and TZ at 60^{o}C for about 24 hrs

Thermal degradation of CH and TZ at 60^{o}C for about 24 hrs in hot air oven was carried out. There were three degradation peak was found at 0.9, 1.0 and 1.3 min for CH in drug product. Degradation of TZ was found out at 0.9 min in both TZ-API and drug product. %Degradation of CH and TZ was found to be 0.87% and 0.98% respectively (Figure 7).

3.5 Photolytic Degradation study of CH and TZ

Sample and drug substances were exposed to energy of 1.2 million lux hrs fluorescent light and 200W/m^2 of UV for about 7 days. There were minor degradation peaks found at 0.9 min and 1.0 min for CH in drug substance and drug product. Degradation peak for TZ was found at 1.4 min. %degradation of CH and TZ was found at 10.11% for CH and 11.95% for TZ. CH showed least degradation in photolytic condition.(Figure 8).

IV. CONCLUSION

Stability indicating RP-UPLC methods for estimation of Ciprofloxacin HCl and Tinidazole in their solid dosage form was established and validated as per the ICH guidelines. The forced degradation study and peak purity data confirmed that there was no merging between peaks of active ingredients and any other degradation products as well as other additives. Hence the specificity of the proposed method was established. The linearity of developed method was achieved in the range of 3.125-43.75 µg/ml for Ciprofloxacin HCl (r^2=0.9999) and 3.75-52.5 µg/ml for Tinidazole (r^2=0.9999). The percentage recovery of drug was achieved in the range of 98-101% which was within the acceptance criteria. The percentage RSD was NMT 2 % which proved the precision of the developed method. Different degradation products were found for drug substance and drug product in acidic, alkaline, oxidative, thermal and photolytic force degradation. Peak of Degraded products were not interfering with the main drug peak of Ciprofloxacin HCl and Tinidazole. Thus these degradation products have not been identified. The developed method is simple, sensitive, rapid, linear, precise, rugged, accurate, specific, and robust. Hence it can be used for the routine analysis of Ciprofloxacin HCl and Tinidazole in their bulk and tablet dosage form in quality control laboratory and stability studies.

V. ACKNOWLEDGEMENT

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Table 1: Regression analysis data and summary of validation parameter for the proposed RP-UPLC method

Parameters	**Acceptance Criteria**	**Ciprofloxacin HCl**	**Tinidazole**
Linearity Range (µg/ml)		3.125-43.75	3.75-52.5
Slope		10935	4388
Intercept		-12303	973.7
Correlation coefficient	> 0.995	0.9999	0.9999
Regression equation y = mx+c		Y = 10935x-12303	Y=4388x+973.7
LOD[a] (µg/ml)	S/N > 2 or 3	0.063	0.01
LOQ[b] (µg/ml)	S/N > 10	0.193	0.0304
Accuracy		99.77%	99.75%
Repeatability (% RSD, n = 6)		0.8%	0.4%
Precision (%RSD)			
Intraday (*n* = 3)		0.12-0.67%	0.13%-0.87%
Interday (*n* = 3)		0.12-0.87%	0.12-0.82%
Accuracy	Recovery 98-102% (individual)	98.7%-99.2% and	99.5%-99.7%.
Specificity	No interference from blank, placebo and degradation product and stress sample	No interference	No interference
	Peak purity index > 0.999	No Purity Error	No Purity Error
% Assay	-	99.12%	97.21%

a=Limit of Detection, b=Limit of Quantitation, c=relative standard deviation

Table 2: Recovery data for the proposed method

Levels	Test Solution µg/mL		Solution added in µg/mL		Total amount of drug inµg/mL		Amount Recovered		% Amount Recovered		% Mean Recovered		% RSD	
	CH	TZ	CH	TZ	CH	TZ	CH	TZ	CH	TZ	CH	TZ	CH	TZ
80%	25	30	20	24	45	54	44.6	53.8	99.1	99.6	98.7	99.7	0.27	0.5
	25	30	20	24	45	54	44.3	53.6	98.4	99.2				
	25	30	20	24	45	54	44.4	53.1	98.6	100.3				
100%	25	30	25	30	50	60	49.6	59.7	99.2	99.5	99.6	99.7	0.6	0.4
	25	30	25	30	50	60	49.7	60.1	99.4	100.2				
	25	30	25	30	50	60	50.2	59.8	100.4	99.6				
120%	25	30	30	36	55	66	54.8	65.3	99.6	98.9	99.2	99.5	0.3	0.72
	25	30	30	36	55	66	54.6	65.6	99.27	99.3				
	25	30	30	36	55	66	54.4	66.2	98.9	100.3				

d=Standard deviation

Table 3: Data for system suitability test for CH and TZ in different degradation condition

Sr. No.	System Suitability Test	Observed Values									
		Degradation									
		Alkaline		Acidic		Oxidative		Photolytic		Thermal	
		CH	TZ	CH	TZ	CH	TZ	CH	TZ	CH	TZ
1	Resolution (Rs)		6.15		5.49		6.07		5.75		5.98
2	Number of plates (N)	9428	11084	9425	11354	9428	10265	9497	11198	9135	11652
3	Tailing factor (T)	1.2	1.05	1.21	1.098	1.3	1.07	1.15	1.02	1.447	1.05

Table 4: % Degradation of CH and TZ in different conditions

Degradation condition	Time	Area		Conc. (µg/ml)		% Potency		% Degradation	
		CH	TZ	CH	TZ	CH	TZ	CH	TZ
Acidic/ 0.1 N HCl/70°C/Reflux/ 4hr/ Solution	0	1059400	432238	24.99	29.98	99.99	99.96	11.98	8.37
	4 hrs	932505	396189	22.0	27.48	88.01	91.62		
Alkaline/ 0.1N NaOH/Reflux/70°C/ 4 hr/ Solution	0	1059401	432400	24.99	29.99	99.99	99.99	17.54	27.72
	4 hrs	873532	312510	20.61	21.68	82.45	72.27		
Oxidative/ 3% H2O2/ Reflux/1 hr/ Solution	0	1058952	432228	24.98	29.98	99.95	99.95	35.0	41.97
	4 hrs	688423	250916	16.24	17.40	64.98	58.02		
Thermal / 60°C/ 24 hr/ Solid	0	1059419	432993	25	30	100	100	0.87	0.98
	24 hrs	1050236	428741	24.78	29.71	99.13	99.02		
Photo/1.2 million lux hrs fluorescent light/200W/m^2 of UV/7 days	0	1059424	432415	25.00	30.00	100	100	10.11	11.95
	7 Days	952248	380728	22.47	26.41	89.88	88.04		

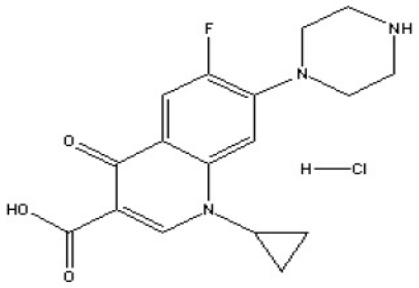

Figure 1: **structure of Ciprofloxacin HCl**

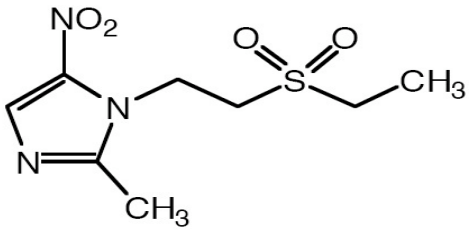

Figure 2**: structure of Tinidazole**

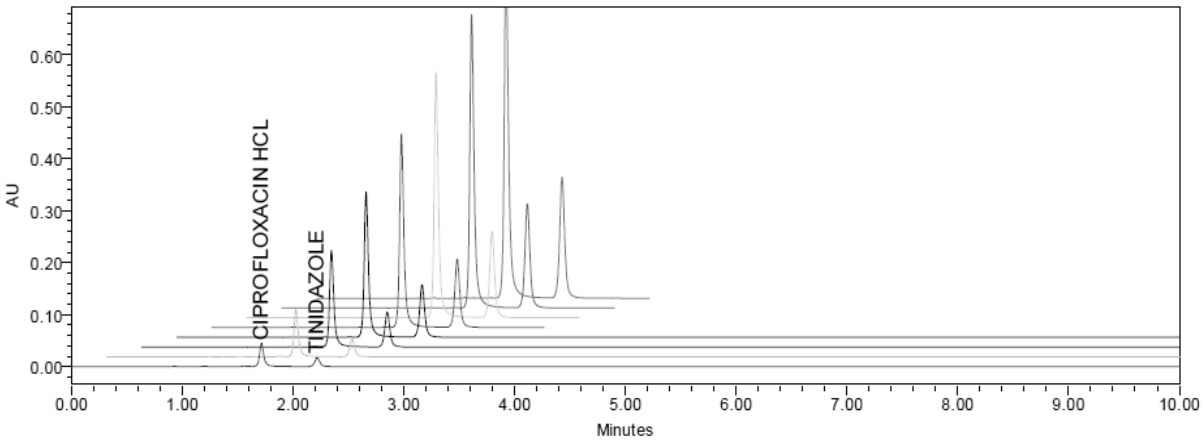

Figure 3: Linearity chromatogram of mixed standard solution of Ciprofloxacin HCl (25µg/ml Rt 1.71 min) and Tinidazole (30µg/ml Rt 2.23 min) by RP-UPLC method.

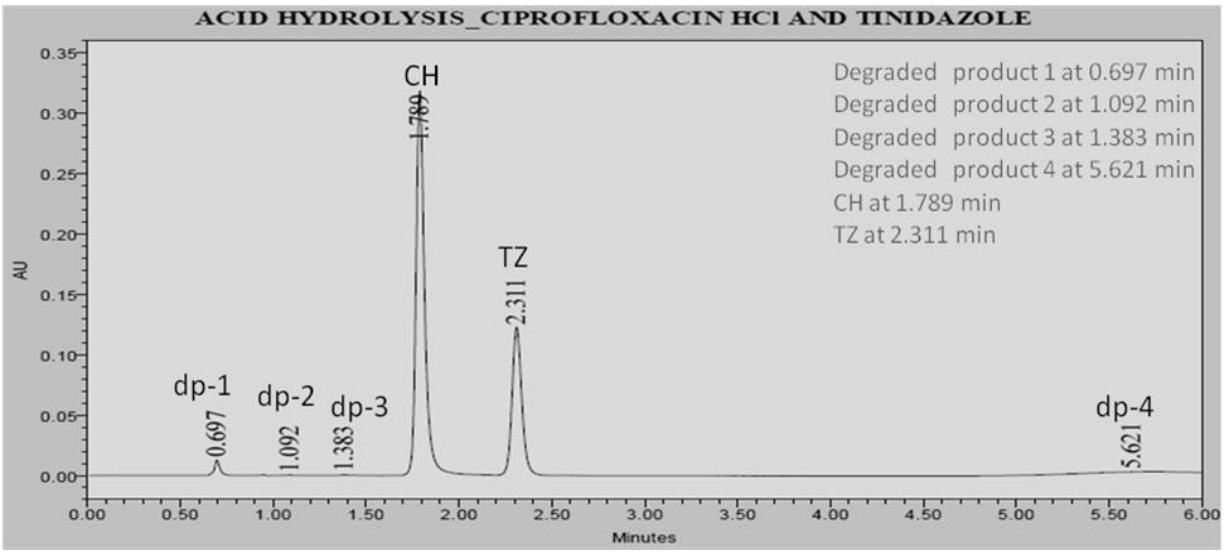

Figure 4: Chromatogram of CH and TZ of drug product after 4 hrs degradation in 0.1 N HCl at 70 ± 2 °C in dark in reflux condition.

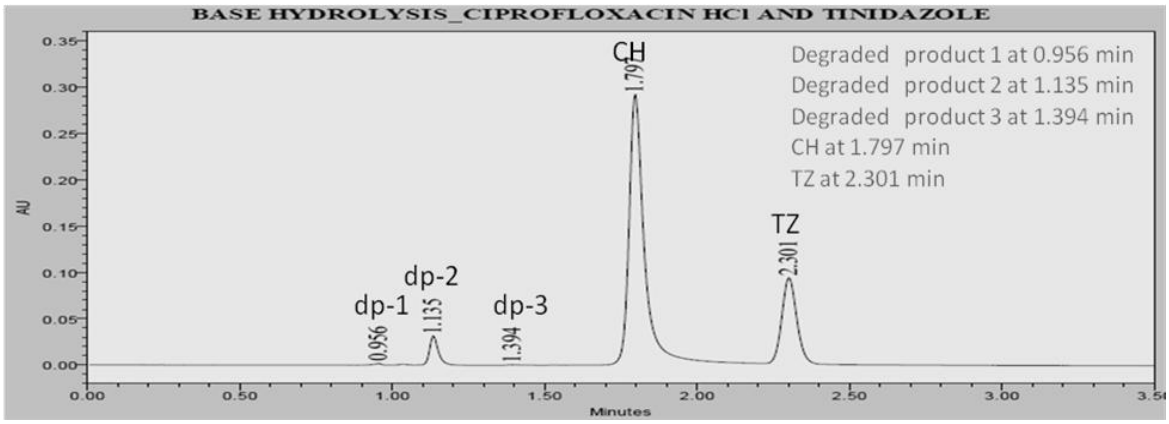

Figure 5: Chromatogram of CH and TZ of drug product after 4 hrs degradation in 0.1 N NaOH at 70 ± 2 °C in dark in reflux condition.

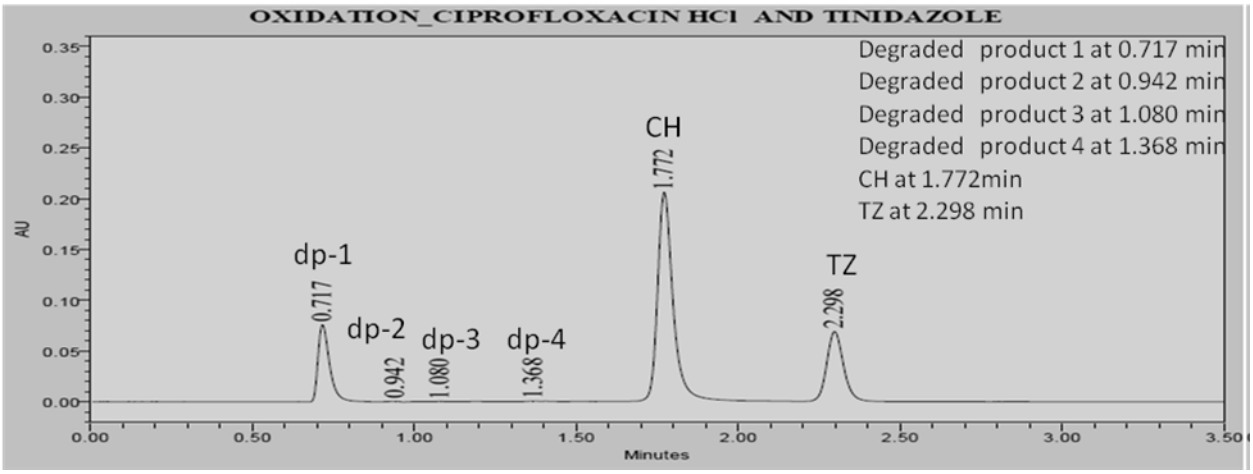

Figure 6: Chromatogram of CH and TZ of drug product after 1 hr degradation in 3 % H_2O_2 at 70 ± 2 °C in dark in reflux condition.

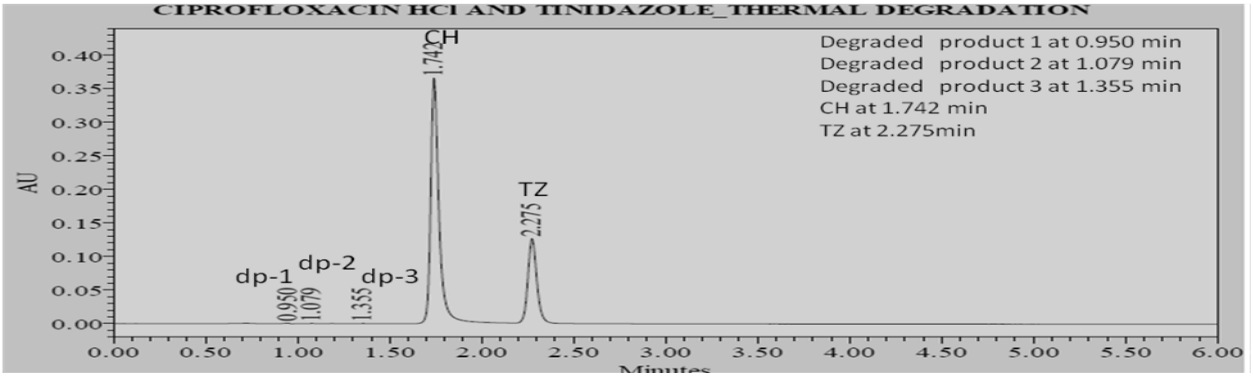

Figure 7: Chromatogram of CH and TZ of drug product at 60°C for about 24 hrs

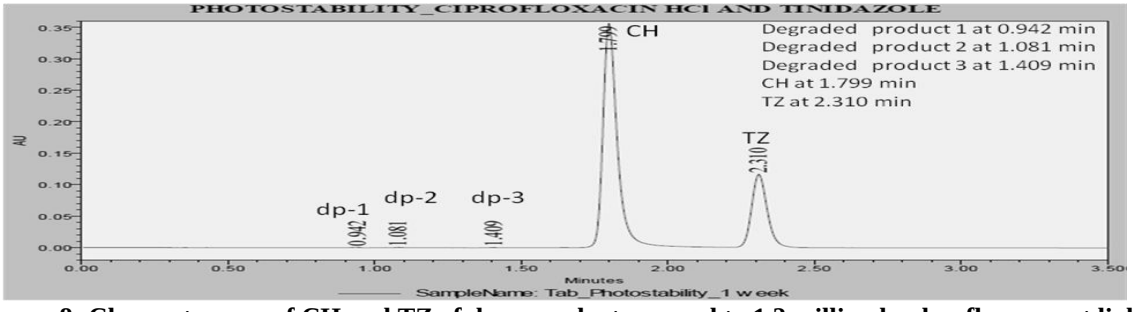

Figure 8: Chromatogram of CH and TZ of drug product exposed to 1.2 million lux hrs fluorescent light and 200W/m2 of UV for about 7 days.