Research Article

Stability Indicating RP-HPLC Method Development and Validation of Ciprofloxacin

Singh Raghabaendra Narayan*, Sahoo Shisir, Mishra Umashankar, Garnaik Bamakanta, Sahoo Sudhir Kumar and Hati Deepak

Laboratories of Royal College of Pharmacy and Health Sciences, Berhampur-760 002, Orissa, India.

ABSTRACT

A stability indicating high performance liquid chromatographic method has been developed for the determination of ciprofloxacin. Optimum separation was achieved in less than 10 minutes using Phenomenex ODS C_{18} (250x 4.6mm packed with 5µ) column. The analyte was resolved by using a mobile phase 20 mmol L^{-1} ammonium formate and acetonitrile (70:30) pH adjusted to 4.0 with formic acid at flow rate 1 ml/min on a high performance liquid chromatographic system at a wavelength of 280 nm. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision, ruggedness and robustness and can be successfully applied for determination of this drug in commercial tablets. For stress studies the drug was subjected to acid, alkali and neutral hydrolysis, oxidation, photolytic and thermal degradation. The degradation studies indicated the drug to be susceptible to acid, alkali hydrolysis and oxidative degradation. The analytical conditions and solvent developed provided good resolution within a short analysis time and economic advantages. The proposed method not required highly sophisticated and expensive instrumentation.

Keywords: Ciprofloxacin, RP-HPLC, Validation, Stability, Degradation.

INTRODUCTION

Ciprofloxacin is the first generation fluoroquinolone antibacterial drug. It was available in market for the treatment of urinary tract infection for many years. Chemically, it is 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid[1-4]. The bactericidal action of ciprofloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair and recombination[5]. The mechanism of action of fluoroquinolones, including ciprofloxacin, is different from that of penicillins, cephalosporins, aminoglycosides, macrolides, and tetracyclines; therefore, microorganisms resistant to these classes of drugs may be susceptible to ciprofloxacin and other fluoroquinolones. There is no known cross-resistance between ciprofloxacin and other classes of antimicrobials. In vitro resistance to ciprofloxacin develops slowly by multiple step mutations. Resistance to ciprofloxacin due to spontaneous mutations occurs at a general frequency of between 10^{-9} to $1x10^{-66}$.

Several analytical methods for ciprofloxacin have been described in scientific literature, books and guide such as UV spectrophotometry, liquid chromatography etc, amongst others[7-12]. The high performance Liquid Chromatography (HPLC) has become an important tool for the routine determination of anti microbial drugs, with specific emphasis on fluoroquinolones, in various animal products, biological fluida, pharmaceutical products, with emphasis on fluoroquinolones[13-18].

In the literature there are some references about the determination of ciprofloxacin using HPLC methodology. Lolo et al. developed a new method for the analysis of the ciprofloxacin in eggs using a diphasic dialysis procedure as extraction and purification method. High pressure liquid chromatography-mass spectrometry (HPLC-MS) was used for the confirmatory determination of this compound[19]. Muchohi et al. described Clinical pharmacokinetic studies of ciprofloxacin require accurate and precise measurement of plasma drug concentrations. And describe a rapid, selective and sensitive HPLC method coupled with fluorescence detection for determination of ciprofloxacin in human plasma[20]. Watabe et al. determined simultaneously three fluoroquinolones in spiked human serum by high-performance liquid chromatography (HPLC) method with fluorescence detection[21]. Faouzi developed a

rapid isocratic technique was developed for the analysis of fluoroquinolones ciprofloxacin in parenteral solutions using high-performance liquid chromatography (HPLC) with fluorimetric detection and a C18 column[22-24].
Our investigation involved the optimization of the method described above using a reliable stability indicating and one new development, as wel as validating a simple, sensitive accurate and reproducible HPLC method for the determination of ciprofloxacin in bulk.

EXPERIMENTAL

CHEMICALS & REAGENTS

Analytically pure Ciprofloxacin was obtained as a gift sample from Aurobindo Pharma, Hyderabad, India. Commercial tablet formulations were purchased from the local market. All chemicals and reagents used were of AR/HPLC grade, obtained from Merck, Qualigens and Loba Chemie.

Instrument

A High Performance Liquid Chromatographic system, with Spinchrom data handling system (Shimadzu-LC 20AT) with Analytical Column-Phenomenex ODS C18 (250 X 4.6 mm, 5 micron particle size), equipped with SPD20A UV-VIS detector in isocratic mode was used for the analysis. Calibrated electronic single pan balance (Sigma 200/A Super), pH Meter (Thermo Fisher scientific), RK 102 CH Iiter 3,0 Ultrasonicator were also used during the analysis.

Chromatographic conditions

A reverse phase C-18 column was equilibrated with the mobile phase Ammonium formate: Acetonitrile (70:30) and pH adjusted 4.0 with formic acid. Mobile phase flow rate was maintained at 1ml/min and eluents were monitored at 280nm for ciprofloxacin. The sample was injected using a 20 µl fixed loop. All determinations were performed at 30^0C for a run time of 10min.

Preparation of mobile phase and Standard Stock Solution

Mobile phase was prepared by mixing 700 ml of 20mM ammonium formate solution with 300 ml of HPLC grade acetonitrile to get the proportion of 70:30 v/v and finally the pH was adjusted to 4.0 with formic acid. The mobile phase was sonicated for 10 minutes and filtered through 0.45µ membrane filter. The standard stock solution of Ciprofloxacin was prepared by dissolving 50mg ciprofloxacin in 50ml of mobile phase to get a concentration of 1000µg/ml volume was made up to the mark.

Calibration curve for Ciprofloxacin

Appropriate aliquots of standard stock solution of the drug was taken in 10 ml volumetric flask and diluted up to the mark with mobile phase in such a way that final concentration of drug was the range of 10-150 µg/ml Ciprofloxacin respectively. The solution was injected using a 20 µl fixed loop system and chromatogram was recorded. Calibration curve was plotted by taking peak area on y-axis and respective concentration of drug on x-axis.

Method Validation[25-27]

1. Linearity

Various working standard solutions were prepared and the linearity range was calculated from the observation.

2. Accuracy

The accuracy of the method was determined by calculating recoveries of drug by method of standard addition. Known amounts of standard drug corresponding to 80%, 100%, and 120% of the label claim was added to pre quantified sample solution, and the amounts of drug were estimated by measuring the peak areas and by fitting these values to the straight line equation of calibration curve.

3. Precision

The intraday and interday precision studies of the drug were carried out by estimating the corresponding responses on the same day and consecutive three days respectively. The results were reported in terms of standard deviation and %RSD.

4. Specificity

The specificity of the proposed RP-HPLC method was determined by complete separation of two peaks with parameters like retention time (R_t), resolution (R_s) and tailing factor (T).

5. Robustness

Robustness of the method was studied by deliberate variations of the analytical parameters such as flow rate (1.0$\pm$0.1 ml/min), concentration of acetonitrile (30$\pm$2%).

6. Ruggedness

Ruggedness is the degree of reproducibility of the results obtained under a verity of conditions, expressed as %RSD. These conditions include different laboratory conditions and different analysts.

7. Sensitivity

The sensitivity of the method was determined with respect to LOD and LOQ. Calibration curves were plotted by using concentration in

the expected detection limit range (0.1-5 µg/ml) for each drug. The standard deviation of y-intercept of regression line was determined and substituted in the following equation for the determination of detection limit and quantification limit. Detection limit = 3.3 σ/s; quantification limit = 10 σ/s; where σ is the standard deviation of y-intercept of regression line and s is the slope of the calibration curve.

Forced Degradation Studies[25-27]

The specificity of the method can be demonstrated through forced degradation studies conducted on the sample using acid, alkaline, oxidative, thermal, photolytic, and UV degradations. The sample was exposed to these conditions and the main peak was studied for the peak purity, thus indicating that the method effectively separated the degradation products from the pure active ingredient.

1. Degradation in Neutral Condition

About 10mg of pure drug was accurately weighed and taken in a 10ml volumetric flask and dissolved in minimum volume of acetonitrile. Then the volume was made up to the mark with water and kept at 70^{0}C. At different time interval solutions were prepared and 20 µl of the sample solution was injected into the HPLC system.

2. Degradation in Acidic Condition

About 10mg of pure drug was accurately weighed and taken in a 10ml volumetric flask and dissolved in minimum volume of acetonitrile. Then the volume was made up to the mark with 1N HCl and kept at 70^{0}C. At different time interval solutions were prepared and 20 µl of the sample solution was injected into the HPLC system.

3. Degradation in Basic Condition

About 10mg of pure drug was accurately weighed and taken in a 10ml volumetric flask and dissolved in minimum volume of acetonitrile. Then the volume was made up to the mark with 1N NaOH and kept at 70^{0}C. At different time interval solutions were prepared and 20 µl of the sample solution was injected into the HPLC system.

4. Oxidative Degradation

About 10mg of pure drug was accurately weighed and taken in a 10ml volumetric flask and dissolved in minimum volume of acetonitrile. Then the volume was made up to the mark with 6% w/v H2O2 and kept at 70^{0}C. At different time interval solutions were prepared and 20 µl of the sample solution was injected into the HPLC system.

5. Photolytic Degradation

About 100 mg of pure drug was taken in a clean petridish and exposed to day light. Sampling was done at an interval of 10h, 1week and 2weeks. From this sample, different solutions were prepared and 20 µl of the sample solution was injected into the HPLC system.

6. UV- Degradation

About 100 mg of pure drug was taken in a clean petridish and subjected to UV illumination of 1.2x106 lux hours. Sampling was done at an interval of 12h, 24h, and 48h and from the sample, different solutions were prepared and 20µl of the sample solutions were injected into the HPLC system.

7. Thermal Degradation

About 100 mg of pure drug was taken in three separate clean petridishes and subjected to dry heat at 70^{0}C. Sampling was done at intervals of 10 days, 20 days and 30 days. Solution of the drug was prepared and 20 µl of the sample solution was injected into the HPLC system.

RESULTS AND DISCUSSION

Calibration Curve

The peak areas for the different concentrations (10-150 µg/ml) were recorded at 280 nm. The calibration curve (Figure 2), and the HPLC Overlay Chromatogram (Figure 3) is shown in Table 1.

Accuracy

The percentage recovery was found to be in the range of 99.73% to 100.14% as shown in Table 2.

Precision

From Table 3, the %RSD for precision was found to be 0.41% and 1.15%.

Sensitivity

The LOD was found to be 0.35µg/ml and the LOQ was found to be 1.16µg/ml at 280 nm respectively.

Intraday and Interday Assay

The %RSD for Intraday and Interday Assay were found to be 0.13 to 0.82 and 0.38% to 0.88% respectively. Low values of %RSD indicate that the proposed method is accurate. The data is shown in Table 4 and 5.

Ruggedness

To evaluate ruggedness of the developed method, deliberate variations were made in the method parameters such as analysts and temperature of the system. The results are found to be %RSD of 0.82 to 0.84 as presented in Table 6.

Robustness

To evaluate robustness of the developed method, deliberate variations were made in the method parameters such as the flow rate of the mobile phase and ratio of mobile phase. The %RSD for different pH was 0.31 to 0.84 and flow rate was 0.05 to 0.16 are presented in Table 7 and 8.

Stability Results

The results obtained in acidic degradation, alkaline degradation, neutral degradation, thermal degradation, oxidative degradation, photolytic degradation and UV degradation are depicted as chromatograms and given in figure 4, 5, 6, 7, 8, 9 and 10 respectively and represented in Table 9.

CONCLUSION

From the results of method development it is found that the developed method is simple, reliable, sensitive and accurate. The developed RP-HPLC method was found suitable for the analysis of selected drug in bulk and in presence of their respective degradants since the resolution between the drugs with their corresponding degradants is better. The optimized chromatographic condition for the selected drug was a reverse phase C-18 column, mobile phase Ammonium Formate solution: Acetonitrile (70:30) pH adjusted to 4.0 with formic acid, flow rate was maintained at 1ml/min and eluents were monitored at 280nm for ciprofloxacin. The sample was injected using a 20 µl fixed loop. The determination was performed at 30^0C for a run time of 10 min.

The method was found to be fast, simple, reliable, sensitive, economical, accurate and precise. The method was found to be linear within the range of 10mcg/ml to 150mcg/ml with regression coefficient of 0.999. The method was found to be accurate with %recovery within 99.73 to 100.14 for ciprofloxacin with the standard deviation and percentage standard deviations were less than 1. The method was found to be precise according to the repeatability data, intraday precision data and interday precision data with the standard deviation and % relative standard deviation less than 2. The method was rugged and robust with the standard deviation and % relative standard deviation less than 2.

Hydrolytic degradation for ciprofloxacin carried out in different conditions showed 11.79%, 16.25% and 18.69% degradation in neutral, acidic and basic conditions respectively. Oxidative degradation ciprofloxacin in presence of hydrogen peroxide showed 26.34% degradation after 11days. Ciprofloxacin was found to degrade up to 6.5% after 11days of exposure to day light. Degradation carried out in presence of UV light showed 9.95% degradation after 11days. The thermal degradation study showed a degradation of 7.33% at70^0C.

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Table 1: Calibration Table of Ciprofloxacin for RP-HPLC Method

Conc. (µg/ml)	PA1	PA2	PA3	PA4	PA5	PA6	Mean	Stdev	%Rsd
10	452.722	458.982	456.812	457.739	455.401	453.723	455.897	2.40	0.53
20	954.242	952.084	955.765	954.391	952.693	954.736	953.985	1.36	0.14
30	1348.82	1349.09	1349.2	1348.3	1346.56	1349.47	1348.57	1.06	0.08
40	1790.45	1789.89	1783.38	1791.21	1789.28	1790.33	1789.09	2.87	0.16
50	2255.45	2258.88	2252.79	2259.45	2256.32	2255.23	2256.35	2.48	0.11
60	2818.32	2814.44	2817.92	2815.33	2815.87	2814.03	2815.99	1.78	0.06
70	3317.03	3319.39	3318.39	3318.11	3318.09	3318.78	3318.3	0.79	0.02
80	3816.68	3812.36	3818.03	3817.45	3815.82	3816.01	3816.06	1.90	0.05
90	4223.13	4222.89	4224.55	4224.44	4223.03	4223.75	4223.63	0.73	0.02
100	4668.66	4667.09	4667.71	4668.01	4661.78	4668.74	4667	1.63	0.06
150	6894.64	6897.22	6894.11	6893.07	6894.32	6899.05	6895.4	1.65	0.03
							Avg	1.85	0.11

Table 2: Accuracy Data of the RP-HPLC Method for Ciprofloxacin

No. of preparations	Concentration (µg/ml)		% Recovery	Statistical Analysis
	Formulation	Pure Drug		
S1 : 80 %	30	24	99.178	Mean=99.73 SD=0.48 %Rsd=0.48
S2 : 80 %	30	24	99.995	
S3 : 80 %	30	24	100.016	
S4 : 100 %	30	30	100.219	Mean=99.93 SD=0.32 %Rsd=0.32
S5 : 100 %	30	30	99.589	
S6 : 100 %	30	30	99.991	
S7 : 120 %	30	36	99.993	Mean=100.14 SD=0.12 %Rsd=0.12
S8 : 120 %	30	36	100.201	
S9 : 120 %	30	36	100.212	

Table 3: Precision Data Showing Repeatability of the RP-HPLC Method for Ciprofloxacin

S.No	Concentration	Peak Area	Calc. Amt.	Statistical Analysis
1	30	1398.98	30.1196	Mean=29.85 SD.=0.34 %Rsd=1.15
2	30	1368.46	29.4633	
3	30	1391.44	29.9574	
4	40	1859.26	40.0158	Mean=39.83 SD.=0.16 %Rsd=0.41
5	40	1847.47	39.7623	
6	40	1844.97	39.7086	

Table 4: Intraday Precision Data of the RP-HPLC Method for Ciprofloxacin

S.No	Conc. (µg/ml)	Peak Area1	Peak Area2	Peak Area3	Statistical Analysis
1	30	1381.55	1379.53	1388.11	Mean=29.79 SD=0.038 %Rsd=0.13
2	30	1385.13	1381.34	1381.2	
3	30	1386.83	1384.12	1385.55	
	Mean	1384.5	1381.66	1384.95	
	Calc. Amt.	29.808	29.747	29.818	
1	40	1781.55	1879.53	1838.11	Mean=39.94 SD=0.33 %Rsd=0.82
2	40	1845.13	1881.34	1831.2	
3	40	1846.83	1834.12	1845.55	
	Mean	1864.25	1864.99	1838.29	
	Calc. Amt.	40.123	40.139	39.565	

Table 5: Interday Precision Data of the RP-HPLC Method for Ciprofloxacin

S.No	Conc.(µg/ml)	Day1	Day2	Day3	Statistical Analysis
1	30	1381.55	1399.53	1388.11	Mean=29.91 SD=0.11 %Rsd=0.38
2	30	1385.13	1391.34	1391.2	
3	30	1386.83	1394.12	1385.55	
	Mean	1384.5	1394.99	1388.29	
	Calc. Amt.	29.8083	30.0339	29.8897	
1	40	1777.49	1812.28	1838.02	Mean=39.71 SD=0.35 %Rsd=0.88
2	40	1855.72	1843.93	1851.62	
3	40	1833.81	1831.68	1842.12	
	Mean	1861.81	1829.3	1843.92	
	Calc. Amt.	40.0707	39.3717	39.6861	

Table 6: Ruggedness Data of the RP-HPLC Method by Different Analysts for Ciprofloxacin

Analyst-1				Analyst-2			
Conc. (µg/ml)	Peak Area	Calc. Amt.	Statistical Analysis	Conc. (µg/ml)	Peak Area	Calc. Amt.	Statistical Analysis
30	1401.3	30.17	Mean=30.01	30	1398.01	30.10	Mean=29.99
30	1388.6	29.90	SD.=0.14	30	1379.77	29.71	SD.=0.25
30	1391.99	29.97	%Rsd=0.47	30	1401.78	30.18	%Rsd=0.84
40	1859.26	40.02	Mean=39.83	40	1869.13	40.23	Mean=40.06
40	1847.47	39.76	SD.=0.16	40	1843.55	39.68	SD.=0.33
40	1844.97	39.71	%Rsd=0.41	40	1871.04	40.269	%Rsd=0.82

Table 7: Robustness Data of the RP-HPLC Method at Different pH for Ciprofloxacin

pH-3.9				pH-4.1			
Conc. (µg/ml)	Peak Area	Calc. Amt.	Statistical Analysis	Conc. (µg/ml)	Peak Area	Calc. Amt.	Statistical Analysis
30	1398.01	30.10	Mean=29.85	30	1395.42	30.04	Mean=29.94
30	1368.75	29.47	SD.=0.33	30	1388.12	29.89	SD.=0.09
30	1391.94	29.97	%Rsd=1.11	30	1387.66	29.88	%Rsd=0.31
40	1859.19	40.01	Mean=40.06	40	1871.03	40.27	Mean=40.10
40	1867.23	40.19	SD.=0.11	40	1873.1	40.31	SD.=0.34
40	1858.12	39.99	%Rsd=0.27	40	1844.99	39.71	%Rsd=0.84

Table 8: Robustness Data of the RP-HPLC Method at Different Flow Rate for Ciprofloxacin

Flow Rate 0.9ml/min				Flow Rate 1.1ml/min			
Conc. (µg/ml)	Peak Area	Calc. Amt.	Statistical Analysis	Conc. (µg/ml)	Peak Area	Calc. Amt.	Statistical Analysis
30	1398.06	30.29	Mean=30.00	30	1398.98	30.12	Mean=30.15
30	1389.91	29.92	SD.=0.09	30	1399.64	30.13	SD.=0.05
30	1391.99	29.97	%Rsd=0.30	30	1403.07	30.21	%Rsd=0.16
40	1859.32	40.02	Mean=40.04	40	1861.12	40.06	Mean=40.05
40	1857.41	39.98	SD.=0.08	40	1860	40.03	SD.=0.02
40	1864.64	40.13	%Rsd=0.20	40	1861.92	40.07	%Rsd=0.05

Table 9: Stability Study Results of Ciprofloxacin

Conditions	Conc. (µg/ml)	Time	% Degraded
Acidic Degradation	100	11days	16.25
Alkaline Degradation	100	11days	18.69
Neutral Degradation	100	1 week	11.79
Thermolytic Degradation	100	11days	7.33
Oxidative Degradation	100	11days	26.34
Photolytic Degradation	100	11days	6.5
UV Degradation	100	11days	9.95

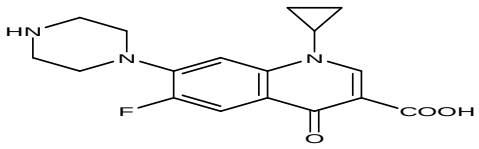

Fig. 1: Chemical Structure of Ciprofloxacin

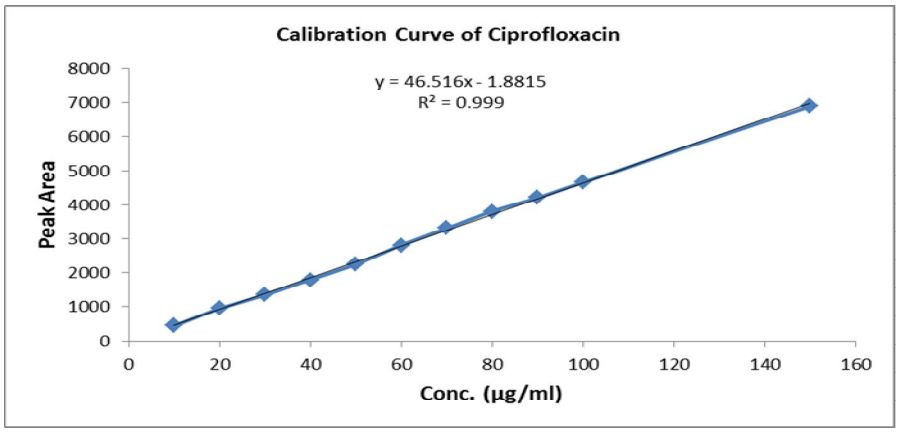

Fig. 2: Calibration Curve of Ciprofloxacin

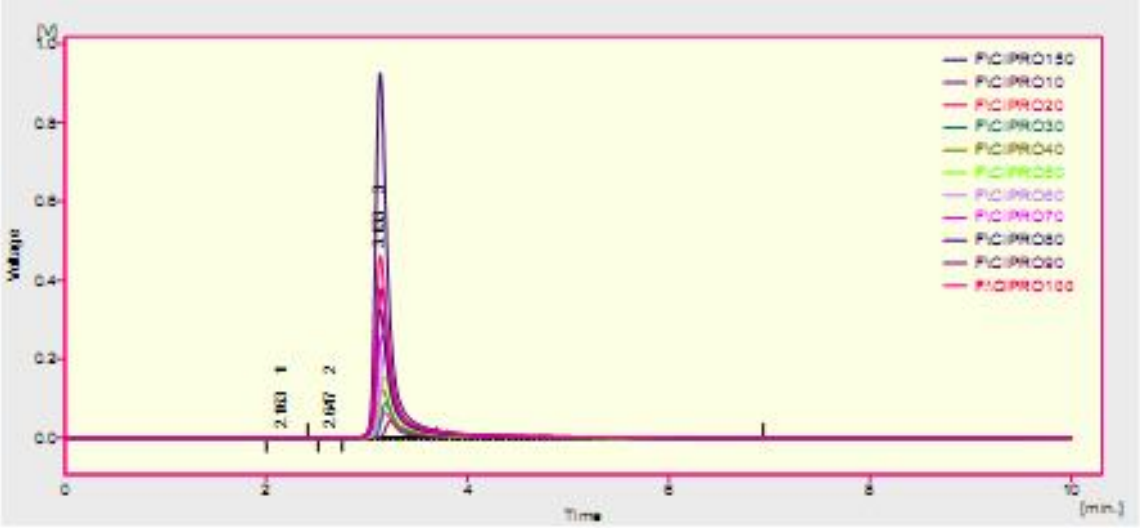

Fig. 3: Typical HPLC Overlay Chromatogram of Ciprofloxacin

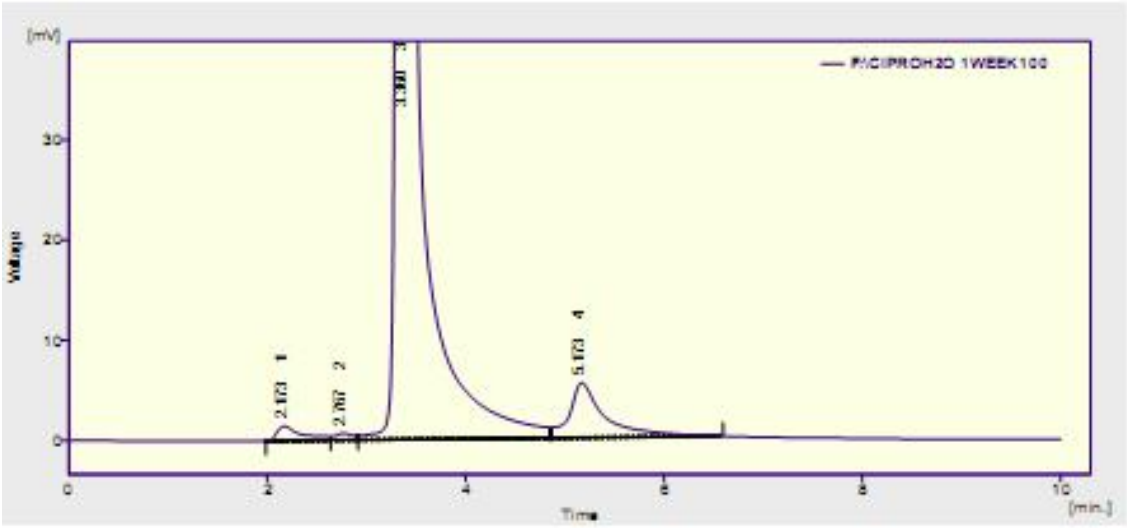

Fig. 4: Representative Chromatogram of Hydrolytic Degradation of Ciprofloxacin

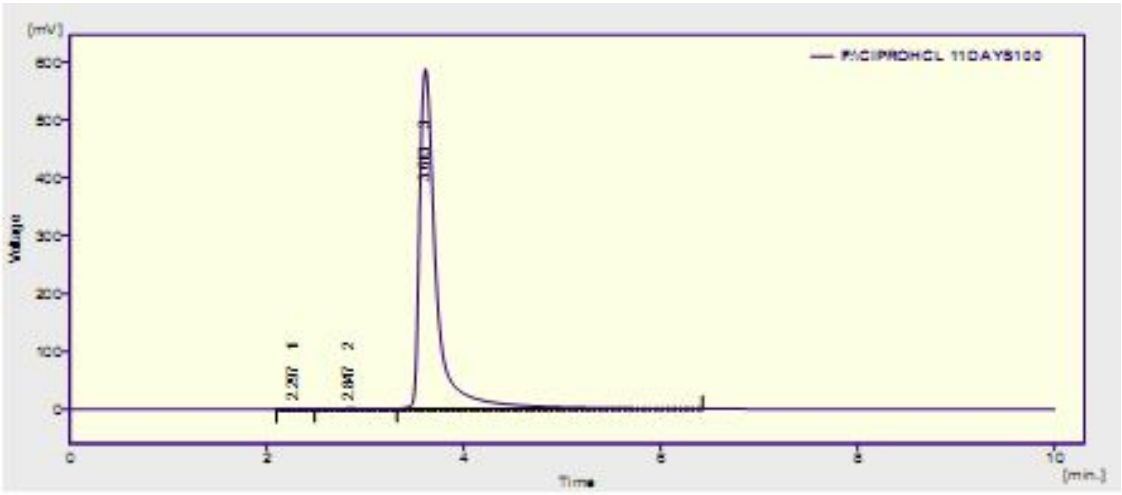

Fig. 5: Representative Chromatogram of Acidic Degradation of Ciprofloxacin

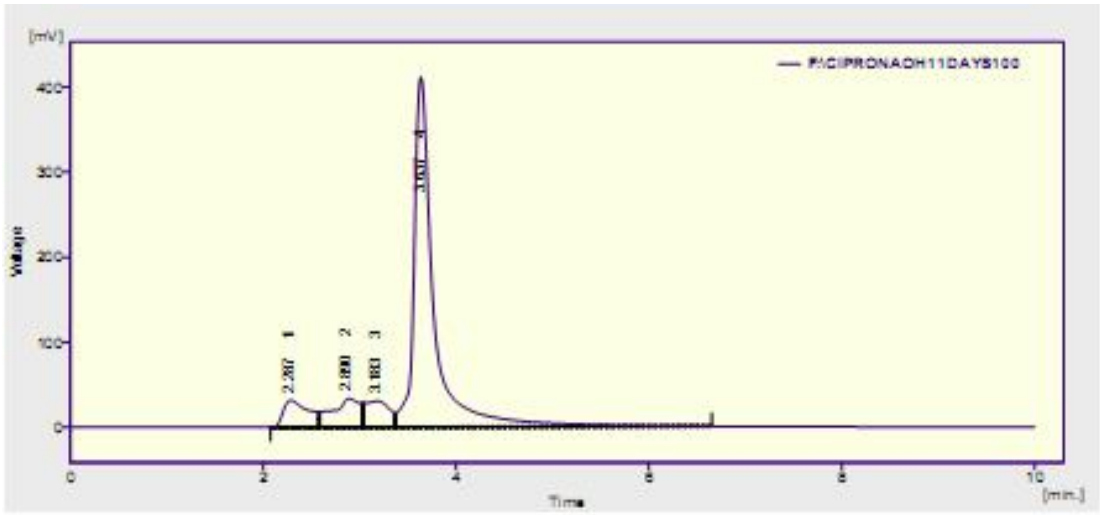

Fig. 6: Representative Chromatogram of Basic Degradation of Ciprofloxacin

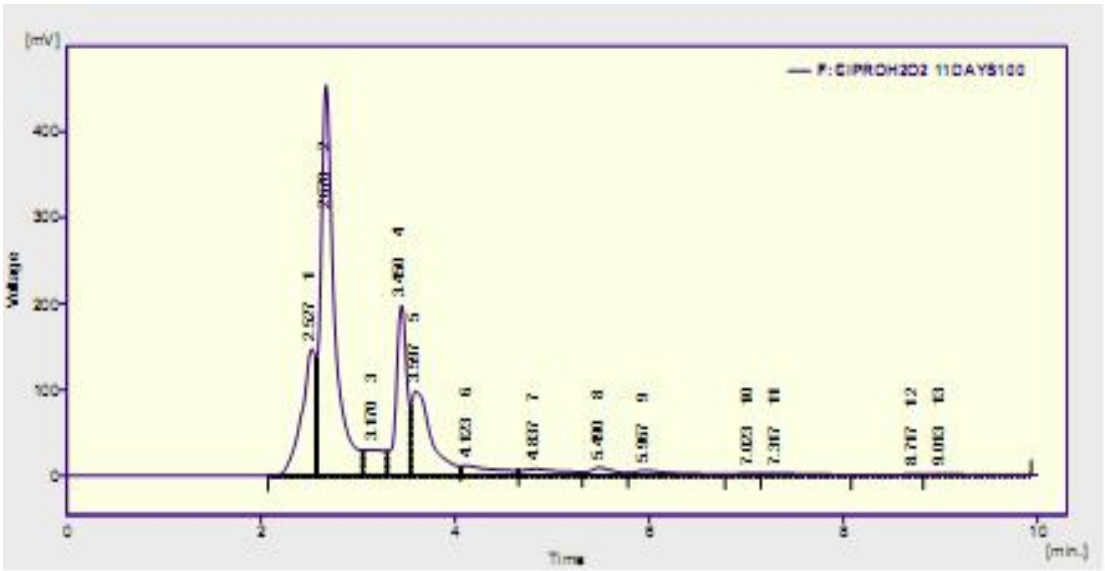

Fig. 7: Representative Chromatogram of Oxidative Degradation of Ciprofloxacin

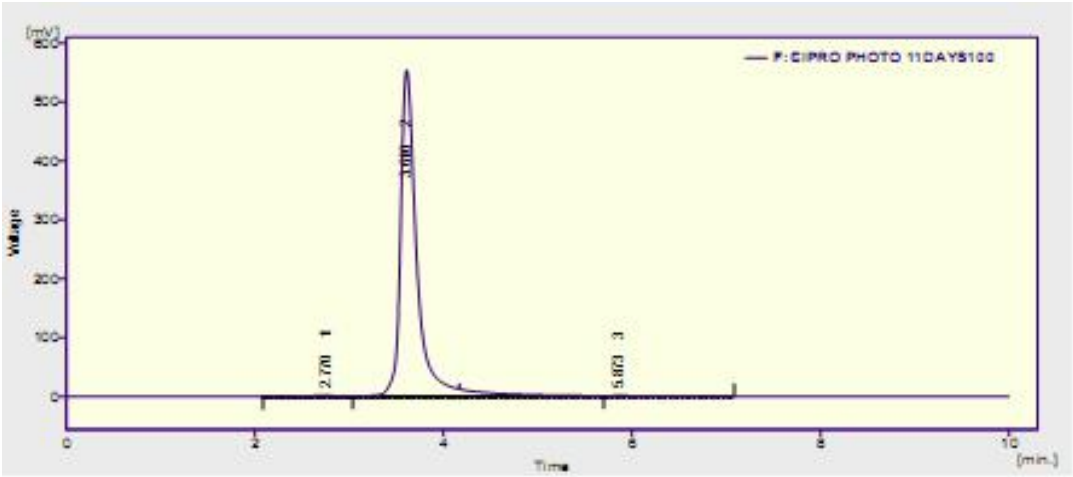

Fig. 8: Representative Chromatogram of Photolytic Degradation of Ciprofloxacin

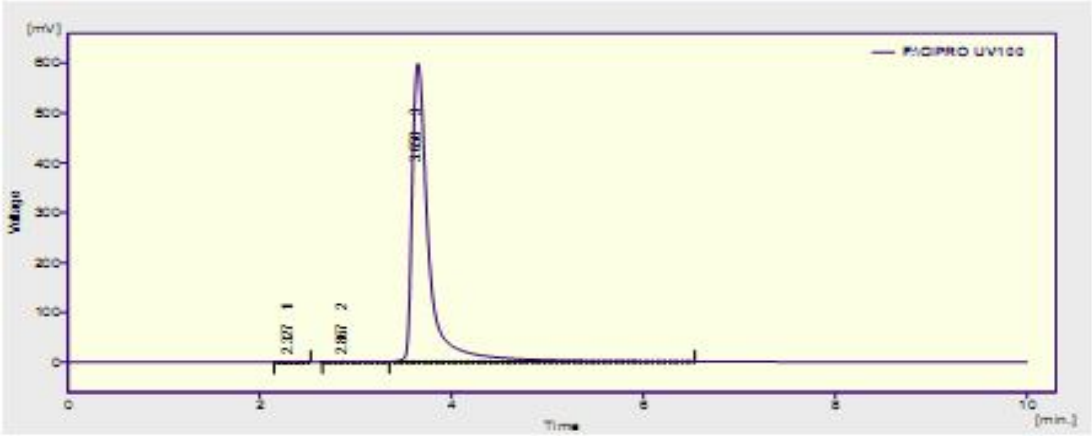

Fig. 9: Representative Chromatogram of UV- Degradation of Ciprofloxacin

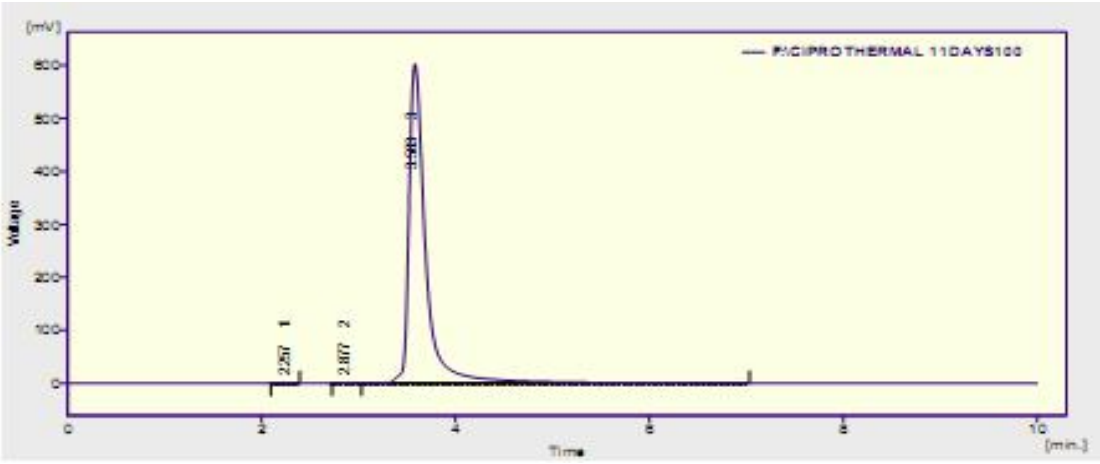

Fig. 10: Representative Chromatogram of Thermal Degradation of Ciprofloxacin

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