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Der Pharma Chemica, 2013, 5(6):132-137  
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ISSN 0975-413X  
CODEN (USA): PCHHAX

## Study of forced degradation of ciprofloxacin HCl indicating stability using RP-HPLC method

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### ABSTRACT

The present study describes the degradation behavior of ciprofloxacin HCl which was investigated under different stress conditions viz acidic, basic, oxidation, UV radiation and thermal conditions. The degradation products were analyzed by HPLC using C-18 column (25 cm × 4.6 mm, 5 μm, Phenomenex Inc.). Forced degradation of the drug product was carried out as per the ICH guidelines. It was found that in alkaline condition i.e. in 0.1N NaOH at 70°C for 4 hours, the degradation of ciprofloxacin HCl was about 24%. In acidic condition (0.1 N HCl at 70°C for 4 h), the degradation was somewhat slower than in alkaline condition and it was found about 20%. In oxidation degradation (3% H<sub>2</sub>O<sub>2</sub> at 70°C for 4 h) of ciprofloxacin HCl was somewhat significant in compare with acidic and alkaline condition and it was found about 40%. The percentage of drug degradation of ciprofloxacin HCl under UV radiation for 5 days and in thermal condition at 60°C for 24hr was about 30% and 10% respectively.

**Keywords:** Ciprofloxacin HCl, stability, forced degradation, validation

### INTRODUCTION

Ciprofloxacin HCl, fluoroquinolone antibacterial agent is widely used to treat a number of infections including: endocarditis, gastroenteritis, malignant otitis externa, respiratory tract infections, urinary tract infections etc<sup>[1]</sup>.

The revised parent drug stability test guideline Q1A (R2) issued by International Conference on Harmonization (ICH) requires that stress testing on the drug substance should be carried out to establish its inherent stability characteristics and for supporting the suitability of the proposed analytical procedures. It is suggested that stress testing should include the effect of temperature, humidity, light, oxidizing agents as well as susceptibility across a wide range of pH values<sup>[2-3]</sup>. It is also recommended that the analyses of stability samples should be carried out by the use of validated stability-indicating testing methods.

Literature survey describes that there is few reported method for degradation studies of Ciprofloxacin HCl, in various stress condition like alkaline, acidic, oxidative, thermal and photo degradation by RP-HPLC method<sup>[4-8]</sup>. This paper deals with the forced degradation of ciprofloxacin under stress conditions like acidic hydrolysis, alkaline hydrolysis, oxidation, UV radiation and thermal stress; and also deals with validation of the developed method for the assay of Ciprofloxacin HCl and its degradation products from its bulk drug and in pharmaceutical dosage forms.

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## MATERIALS AND METHODS

### Drugs and reagents

Working standard of ciprofloxacin HCl with the potency of 99.29% was a kind gift of Eskyef Pharmaceuticals Ltd., Dhaka, Bangladesh. Orthophosphoric acid, HPLC grade acetonitrile and methanol were purchased from Active Fine Chemicals Ltd., Dhaka Bangladesh.

### Instrumentation

High Performance Liquid Chromatographic system (Shimadzu-UFLC Prominence), equipped with an auto sampler (Model- SIL 20AC HT) and UV-Visible detector (Model-SPD 20A) was used for the analysis. The data was recorded using LC-solutions software. Phenomenex C18 (4.6 mm x 250 mm; 5  $\mu$ m) column was used for the analysis.

### Preparation of mobile phase

Accurately Weigh 6.8 gm of Potassium dihydrogen ortho phosphate was transferred into 1000 ml volumetric flask. About 500 mL of double distilled water was added into the flask, dissolved the salt and finally water was added up to the mark. Then pH was adjusted to 3 by adding dilute sodium hydroxide solution. The mixture was sonicated for 10 minutes and then filtered through a 0.22  $\mu$ m millipore filter. HPLC grade acetonitrile was also filtered and degassed before use into the HPLC system.

### Standard preparation

10 mg ciprofloxacin HCl was weighed and transferred into 10 mL volumetric flask containing about 7 mL of mobile phase. The solution was sonicated for 15 min to dissolve the drug completely and the volume made up with mobile phase to get the concentration of ciprofloxacin HCl of 1 mg/mL solution. Further dilution was carried to get concentration of 10, 20, 30, 40, and 50  $\mu$ g/mL of ciprofloxacin HCl.

### Sample preparation

Twenty ciprofloxacin HCl tablets were weighed and the average weigh was calculated. Sample equivalent to 10 mg of ciprofloxacin HCl was weighed and transferred into 10 mL volumetric flask containing 7 mL of mobile phase. The solution was sonicated for 15 min to dissolve the drug completely and the volume made up to the mark with mobile phase and was filtered through 0.45  $\mu$ m filter and it was further diluted.

### Chromatographic conditions

For quantitative analysis of ciprofloxacin HCl by RP-HPLC method, the mobile phase was comprised of Potassium dihydrogen ortho phosphate (pH 3) and acetonitrile in the ratio of 80:20 (v/v) at a flow rate of 1 mL/min. The injection volume was 20  $\mu$ L for both standard and samples. The run time was set for 15 min. Before analysis, every standard and sample was filtered through 0.45  $\mu$ m filter tips. The mobile phase was also filtered, sonicated and degassed before use. The column eluate was monitored with a UV detector at 278 nm. All analyses were done at ambient temperature under isocratic condition.

### Method validation

The methods were validated for different parameters like linearity, accuracy, precision, robustness, LOD, LOQ etc.

### Linearity

The linearity of the developed method was performed with a concentration range of 10, 20, 30, 40 and 50  $\mu$ g/mL by injecting repeated thrice times. The average peak areas were plotted against respective concentration. The linearity of the proposed method was evaluated by using calibration curves to calculate coefficient of correlation and intercept values.

### Accuracy

The accuracy of the method was evaluated by determination of recovery of ciprofloxacin HCl at three levels of concentrations at three times. The sample solutions were spiked with ciprofloxacin HCl standard solutions corresponding to 50%, 100% and 150% of nominal analytical concentrations.

**Precision**

The precision of the method was demonstrated by intra-day and inter-day variation studies. In intra-day precision was established by analyzing three replicates over three concentrations (20, 40, 60 µg/mL) of ciprofloxacin HCl. Inter-day precision was carried out by three concentrations with three replicates for consecutive 3 days. The precision was expressed as %RSD amongst responses using the formula [%RSD = (standard deviation/mean) x 100 %].

**Robustness**

Robustness of the proposed method was determined by small deliberate changes in flow rate (0.8, 1, 1.2 mL/min), change in organic composition of mobile phase ratio (±2%).

**LOD and LOQ**

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions for six times.

**System suitability**

A standard solution of ciprofloxacin HCl was prepared as per procedure and was injected three times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms obtained by calculating the %RSD of retention times, tailing factor, theoretical plates and peak area from three replicate injections.

**Forced degradation study of ciprofloxacin HCl**

Forced degradation of the drug product was carried out as per the ICH guideline. The forced degradation study of ciprofloxacin HCl was performed in acidic, alkaline and oxidant media, under UV radiation and thermal conditions.

**Acidic degradation**

1 mg/mL of ciprofloxacin HCl was prepared by dissolving 50 mg of ciprofloxacin HCl in 50 mL of 0.1N methanolic hydrochloric acid. 25 mL of this solution was refluxed in round bottom flask at 70°C in the thermostatically controlled heating chamber. The remaining solution was kept at room temperature. 0.1 mL of the solution was withdrawn at 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup> and 8<sup>th</sup> h and was diluted with mobile phase. Then the samples were analyzed by HPLC to study the extent of degradation.

**Alkaline degradation**

1 mg/mL of ciprofloxacin HCl was prepared by dissolving 50 mg of ciprofloxacin HCl in 50 mL of 0.1N methanolic NaOH and 25 mL of the solution was refluxed in round bottom flask at 70°C in the thermostatically controlled heating chamber. The remaining solution was kept at room temperature. 0.1 mL of the solution was withdrawn at 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup> and 8<sup>th</sup> h and was diluted with mobile phase. Then the samples were analyzed by HPLC to study the extent of degradation.

**Oxidative degradation**

1 mg/mL of ciprofloxacin HCl was prepared by dissolving 50 mg of ciprofloxacin HCl in 50 mL of 3% methanolic H<sub>2</sub>O<sub>2</sub> solution and 25 mL of the solution was refluxed in round bottom flask at 70°C in the thermostatically controlled heating chamber. The remaining solution was kept at room temperature. 0.1 mL of the solution was withdrawn at 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup> and 8<sup>th</sup> h and was diluted with mobile phase. Then the samples were analyzed by HPLC to study the extent of degradation.

**UV-radiation degradation (at 254 nm)**

About 100 µg/mL of ciprofloxacin HCl solution was exposed to UV radiation at 254 nm. 5 mL of sample solution were taken after 1, 3, 5 days. The solutions were diluted with mobile phase. The samples were analyzed by HPLC to study the extent of degradation.

**Thermal degradation**

Evenly dispersed of 10 mg of ciprofloxacin HCl in a thin layer in the Petridis was kept in the oven at the temperature of 60°C. 1 mg sample was taken at 6, 12, 18 and 24 h and was diluted with mobile phase. Then the samples were analyzed by HPLC to study the extent of degradation.

**RESULTS AND DISCUSSION**

HPLC is of one the most accurate analytical techniques used for qualitative and quantitative determinations of bulk and finished pharmaceutical as well as degraded products. A RP-HPLC method was developed and validated as per ICH, USP and FDA guidelines for quantitative determination of ciprofloxacin HCl after forced degradation by using the mobile phase comprising of Potassium dihydrogen ortho phosphate (pH 3) and acetonitrile in the ratio of 80:20 (v/v) at a flow rate of 1 mL/min, wave length detection at 278nm on C18 column, revealed good resolution and peak shape for ciprofloxacin HCl. The retention time was found to be  $4.8 \pm 0.1$  min. The specificity of the method was monitored. (Fig.1)

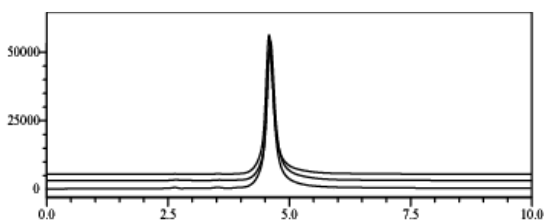


Fig. 1 System Suitability

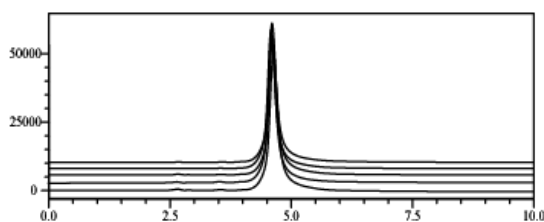


Fig.2 HPLC Chromatogram for Calibration curve

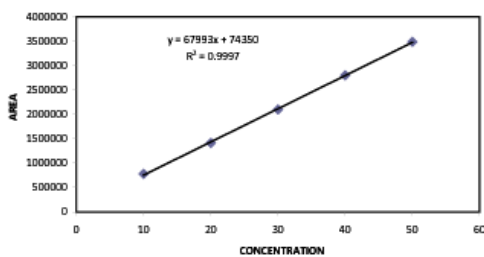


Fig. 3 Calibration curve of ciprofloxacin

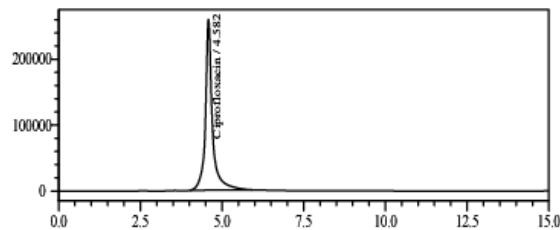


Fig.4 Alkaline degradation

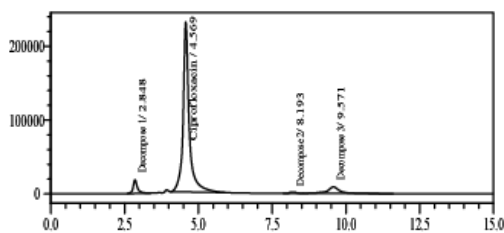


Fig. 5 Oxidation degradation

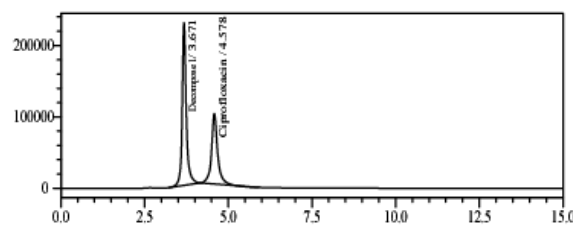


Fig. 6 Acidic degradation

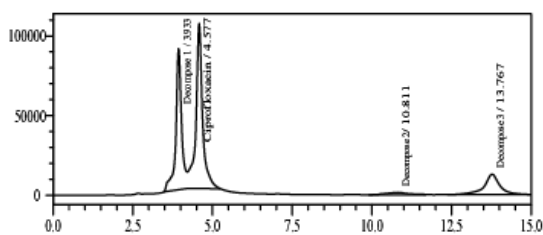


Fig.7 UV degradation

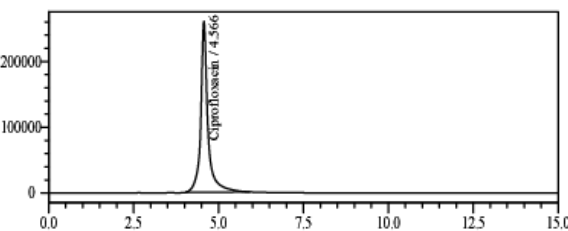


Fig.8 Thermal degradation

**Table 1. Accuracy of ciprofloxacin HCl**

Run	Injected %concentration level	Recovered % concentration level	% Recovery	%Mean
1	50	50.13	100.26	
2		49.99	99.98	100.09
3		50.01	100.02	
1	100	100.08	100.08	
2		99.88	99.88	100.02
3		100.11	100.11	
1	150	150.14	100.09	
2		149.98	99.99	100.02
3		149.96	99.97	

**Table 2. Results for intra-day and inter-day precision**

Inter-day precision			
Concentration ( $\mu\text{g/mL}$ )	Mean Area	SD	% RSD
20	854400	1011	1.12
40	2796484	1431	1.07
60	6147310	2432	1.13
Intra-day precision			
20	848912	1125	1.21
40	2805373	1915	1.25
60	6099321	2221	1.14

**Table 3. Result for robustness study**

Change in flow rate	Retention time	Mean	% RSD
0.8	4.9	2806345	0.98
1.0	4.8	2796484	1.07
1.2	4.6	2699753	1.13
Change in organic composition in the mobile phase			
5% less	4.7	2797983	0.99
Actual	4.8	2796484	1.16
5% more	4.9	2800471	1.13

When average peak areas were plotted against concentration levels of 10-50  $\mu\text{g/mL}$  of standard drug, good correlation coefficient ( $r^2$ ) was obtained as 0.999 which was within the accepted range of guidelines and represented a good linear relationship (Fig. 2 and 3).

The accuracy was evaluated at three different concentrations which were conducted in successive analysis ( $n = 3$ ) using the proposed method and the value was expressed as percentage of recovery between the mean concentrations of recovered and injected concentration of the drug. The average recoveries were found to be as 100.09%, 100.02% and 100.02% for the concentration levels of 50%, 100% and 150%, respectively (Table 1).

The precision of the proposed method was checked by intra-day and inter-day repeatability of responses after replicate injections of standard solutions of different concentrations thrice times each day for three days where RSD % amongst responses were found as  $\leq 2$  (Table 2).

The %RSD was found in the range of 0.98 – 1.16% for robustness and ruggedness (Table 3).

The LOD and LOQ of the developed method were determined by injecting progressively low concentrations of the standard solutions for 6 times and the values of LOD and LOQ were found to be as 0.12  $\mu\text{g/mL}$  and 0.13  $\mu\text{g/mL}$ , respectively. All experimental results were within the range of the acceptability, which indicated that the developed method was sensitive enough and accurate for qualitative, quantitative analysis of ciprofloxacin HCl. Therefore, the method was applied for quantitative analysis of forced degraded products of ciprofloxacin HCl.

The percentage of drug degradation of ciprofloxacin HCl in 0.1N NaOH at 70°C for 4 hours was found 23.63% (Fig. 4). The percentage of drug degradation of ciprofloxacin HCl in 3% solution of  $\text{H}_2\text{O}_2$  at 70°C for 4 hours was found 39.66% (Fig. 5). The percentage of drug degradation of ciprofloxacin HCl in 0.1N HCl at 70°C for 4 hours was

observed 19.24% (Fig. 6). The percentage of drug degradation of ciprofloxacin HCl under UV radiation for 5 days was 28.12% (Fig. 7). The percentage of drug degradation of ciprofloxacin HCl in thermal condition at 60°C for 24hr was found 8.21% (Fig. 8).

### CONCLUSION

Forced degradation of ciprofloxacin HCl in various conditions like alkaline, acidic, oxidation, UV radiation and thermal degradation was observed in this investigation. The content of degradation of the drug was quantitatively analyzed by HPLC. For this purpose a new RP-HPLC method was developed and validated that was also mentioned in this paper. It was found that degradation in alkaline condition was greater than in acidic condition. In oxidation degradation of ciprofloxacin HCl was significant. The drug was also degraded under exposing UV radiation and in the thermal condition.

### Acknowledgement

We express our thanks and gratitude to the centre for Advanced Research in Sciences, University of Dhaka and Department of Pharmacy, Manarat University for logistic support to conduct this research.

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