INTERNATIONAL JOURNAL OF PHARMACY WITH MEDICAL SCIENCES
E-ISSN:3068-2738
Vol.4, No. 4(2024)
www.ijpams.com

ANALYTICAL AND BIOLOGICAL ASSESSMENT OF CIPROFLOXACIN HYDROCHLORIDE UNDER FORCED DEGRADATION CONDITIONS

Sarmin Siraj Sormy
Department of Medicinal Chemistry
Sechenov First Moscow State Medical University, Moscow, Russia

ABSTRACT

Forced degradation studies are essential to evaluate the stability profile and identify potential degradation pathways of pharmaceutical compounds. Ciprofloxacin hydrochloride, a widely used fluoroquinolone antibiotic, was subjected to forced degradation under stress conditions including hydrolytic, oxidative, photolytic, and thermal environments. The degradation behavior was investigated using UV–Visible spectrophotometry and further validated by microbiological assay against selected bacterial strains. UV spectrophotometric analysis provided quantitative data on drug degradation by monitoring absorbance changes at characteristic wavelengths, while microbiological assay confirmed the impact of degradation on antimicrobial potency. Results revealed significant susceptibility of ciprofloxacin hydrochloride to oxidative and photolytic stress, leading to reduced drug stability and diminished antibacterial activity. Hydrolytic and thermal degradation exhibited moderate effects. The combined analytical and microbiological approach provided a comprehensive evaluation of both chemical stability and biological efficacy. These findings emphasize the importance of forced degradation studies in ensuring drug quality, safety, and therapeutic reliability throughout storage and handling.

1. INTRODUCTION

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

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 [2-3]. 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 [4-8] . 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.

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),

Received: 06-11-2024 | Accepted: 19-12-2024 | Published: 26-12-2024 |

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 µm) 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 µm millipore filter. HPLC grade actonitrile 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 µg/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 µm 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 µL for both standard and samples. The run time was set for 15 min. Before analysis, every standard and sample was filtered through 0.45 µm 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.

2. 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 µg/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%).

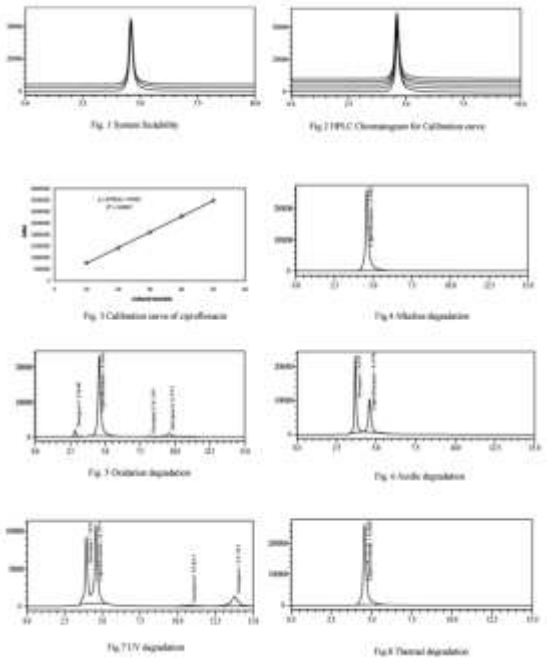
Received: 06-11-2024 | Accepted: 19-12-2024 | Published: 26-12-2024 |

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.

3. 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+ 0.1 min. The specificity of the method was monitored. (Fig.1)



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+ 0.1 min. The specificity of the method was monitored. (Fig.1).

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

Concentration (µg/mL)	Mean Area	SD	% RSD
Inter-day precision			
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 µg/mL of standard drug, good correlation coefficient (r2) 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 ≤ 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 µg/mL and 0.13 µg/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 700C for 4 hours was found 23.63% (Fig. 4).The percentage of drug degradation of ciprofloxacin HCl in 3% solution of H2O2 at 700C for 4 hours was found 39.66% (Fig. 5).The percentage of drug degradation of ciprofloxacin HCl in 0.1N HCL at 700C 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 600C for 24hr was found 8.21% (Fig. 8).

4. CONCLUSION

The study demonstrated that ciprofloxacin hydrochloride undergoes substantial degradation under oxidative and photolytic conditions, with corresponding loss of antimicrobial activity confirmed through microbiological assay. UV–Visible spectrophotometry proved to be a simple, reliable, and cost-effective technique for monitoring drug degradation, while microbiological assays provided valuable insights into the biological consequences of chemical breakdown. Together, these methods offer a robust framework for stability testing of antibiotics. The outcomes highlight the need for stringent storage conditions and protective packaging to preserve the stability and therapeutic effectiveness of ciprofloxacin hydrochloride. Future studies may focus on identifying degradation products and their toxicological implications, ensuring complete safety and efficacy of the drug in clinical use.

REFERENCES

[1] Sean C Sweetman, Martindale, Thirty sixth edition, Royal Pharmaceutical Society of Great Britain (RPS) Publishing, UK, 2009, p-243.

[2] International Conference on Harmonization (ICH), Stability Testing New Drug Substance and Drug Products, Q1A [R2], 2003.

[3] International Conference on Harmonization (ICH), J. Chem. Educ, 1961, 38,559-560.

[4] Bhatia M.S, Kaskhedikar S.G, Chaturvedi S.C, International Journal of Pharmaceutical sciences, SeptemberOctober 1999, pp 311-312.

[5] Sani A.A, Chijioke C. and Mohammed Ilyas, Journal of Applied Pharmaceutical Science, 2011, 1 (8), 239-243.

[6] Sneha Jansari K, Nirav Patel B, Parag Patel R, Nikita Patel N, Henant Desai T, IOSR Journal of Pharmacy, sepoct 2012, pp. 12-19.

[7] Elsadig H.K. Adam, Mohammed EI Mukhtar Abd Elaziz and Ahmed E. M. Saeed, Der Pharmacia Sinica, 2012, 3 (2):217-223.

[8] Sharma R, Pathodiya G, Mishra G. P, Sainy J, Journal of Pharmacy Research, 2011, 4(3), 859-861.