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Stability study of ciprofloxacin hydrochloride under stress conditions using reverse phase -high performance liquid chromatography method

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ABSTRACT

The present study describes behavior of ciprofloxacin hydrochloride (Cipro-HCl) which was investigated under different stress conditions of sun light, UV light at 254 nm and some pharmaceutical excipients using HPLC. RP-HPLC method were modified that could separate the drug from its degradation products formed under these stress conditions. Degradation was found to occur under sun light and thermal, were successfully resolved on a C18 column (5S, ODS 25 cm × 4.6 mm, 5µm), utilizing mobile phase of 0.025 M orthophospharic acid (adjusted to pH 3.0 with triethylamine) and acetonitrile in a ratio of 87:13 respectively. Mobile phase was delivered at the flow rate of 1.5 ml/min. Ultra violet detection was carried out at 278 nm. The method was validated with respect to linearity, precision, accuracy, selectivity, specificity and ruggedness. The method was specific to drug and also selective to degradation products. Some pharmaceutical excipinents were found to decrease the reaction rate of sunlight and thermal decomposition of ciprofloxacin hydrochloride in aqueous media. Other pharmaceutical excipinents were found to increase the reaction rate of ciprofloxacin hydrochloride sun and thermal decomposition.

Keyword: Ciprofloxacin hydrochloride, *Stability, Thermal degradation, Sunlight degradation, pharmaceutical excipients.*

INTRODUCTION

Ciprofloxacin hydrochloride is basically 1-cyclopropyl-6-fluro-1, 4-dihydro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid hydrochloride. Its empirical formula is $C_{17}H_{18}FN_3O_3$, HCl .The molecular weight of which is 367.8 [1].

Ciprofloxacin hydrochloride is a fluoroquinoline antibacterial agent with a wide spectrum of activity [2].

Degradation reaction in pharmaceutical formulations take place at definite rates and are chemical in nature .They depend on such conditions as concentration of reactants, temperature, pH, radiation, and catalysts. The common stress conditions include acidic pH, basic pH, neutral pH, different temperature and humidity conditions, oxidation, reduction and photo-degradation [3].

These studies help to determine the significant related substances to be used in method development [4], and to determine the degraded product formed under stress conditions, and the effect of pharmaceutical excipients on the reaction rate of ciprofloxacin hydrochloride [3,4].

The present study aimed at investigation of the photo-thermal stability of ciprofloxacin hydrochloride

MATERIALS AND METHODS

Chemicals and Reagents:
All chemicals and reagents used were of a HPLC grade. Acetonitrile HPLC grade was obtained from BDH Labs, England.Orthophosphoric acid 85 % was obtained from BDH laboratory, England. Triethylamine GPR^{TM} was obtained from BDH laboratory, England**.**

Ciprofloxacin hydrochloride was kindly supplied from Dr .Reddy's laboratories ltd –India

Equipment:
1. HPLC was performed using a PERKIN ELMER HPLC system 200 consisting of LC binary pump series 200; Diode Array Detector 235C, Link (Interface) series 600, Software Turbo chrome and turbo scan programme, and desk Jet exi for windows 660.
2. Column used was C18, S5, ODS 25 cm x 4.6 mm ID, 5.0µm
3. Sartorius model cp224s balance
4. Mi 180 Bench pH meter, MARTINI instruments.

Preparation of mobile phase
87 volumes of 0.025M phosphoric acid (adjusted with triethylamine to a pH 3.0 ± 0.1) and 13 volumes of a cetonitrile.

The stock standard solution having concentration of 0.25 mg/ml was prepared by dissolving pure drug of ciprofloxacin hydrochloride in distilled water.

The calibration curve solutions were obtained by further diluted stock solution to get concentrations of 2, 4, 6, 8, and 10µg/ml of ciprofloxacin hydrochloride **(Fig.1).**

Preparation of sun decomposed ciprofloxacin hydrochloride solid

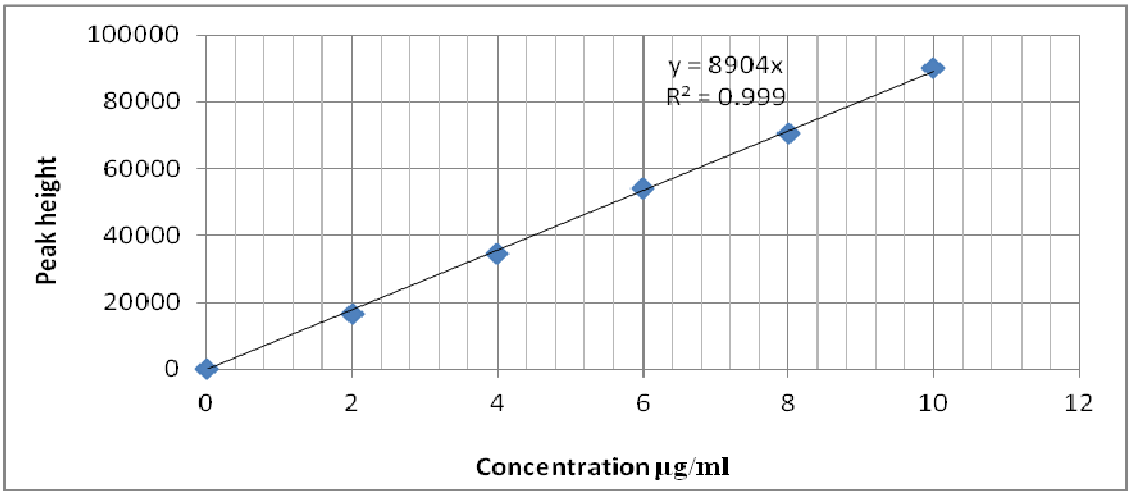

Figure 1. Concentration vs. peak height of standard to illustrate the calibration curve by HPLC

Few grams of ciprofloxacin hydrochloride solid were placed between two glass plates (20 x 20 cm), sealed with gum tape and directly exposed to sunlight for six months (March to August). Samples were taken every month and tested for degradation by RP-HPLC.25µg / ml of sun decomposed ciprofloxacin hydrochloride was prepared. Fifty µl of the sample and the reference standard were separately injected into the chromatographic column.

From the chromatograms obtained, the peak height of the reference standard and the decomposed product were measured, the remaining concentration and reaction rate (k) of ciprofloxacin hydrochloride were calculated **(Table 1)**.

Table (1): The effect of time on the reaction rate of sun- decomposed ciprofloxacin hydrochloride solid

Interval time / month	Reaction rate (k)- month^{-1}
1	0.0871
2	0.1112
3	0.1345
4	0.1483
5	0.1655
6	0.1943

The effect of time on the stability of cefixime trihydrate solution sun decomposition
25 µg/ml water of ciprofloxacin hydrochloride was prepared and directly exposed to sunlight. Samples were taken at interval time at every day and analyzed by RP-HPLC

Table (2): The effect of time on the reaction rate of sun decomposed of ciprofloxacin hydrochloride solution

Time / hr	Reaction rate (k)- hr^{-1}
1	0.0069
2	0.1058
3	0.1107
4	0.1193
5	0.1231
6	0.1332
7	0.1634

The effect of pH- value on the reaction rate of ciprofloxacin hydrochloride sun-decomposition:
Serial buffer solutions with pH 2, 4, 6,8,10, and 12 were prepared according to the method described by Carmody [5].

5 ml of ciprofloxacin hydrochloride solution (25 mg / 100 ml water) were pipette, and separately transferred to six 100-ml volumetric flask and each volume was completed to 100-ml with one of the universal buffer solution mentioned above. Each sample was exposed directly to sunlight.

Ten mls of each sample were taken at 0, 30, 60, 90, 120, and 150 minutes and transferred separately to a small beaker and cooled with iced water; 5 ml for these solutions were transferred to 25 ml volumetric flasks, and the volume was completed to the mark with the mobile phase **(Table 3)** .

Table (3): The effect of pH-value on the reaction rate of sun-decomposed ciprofloxacin hydrochloride

pH - value	Reaction rate (k) -min^{-1}
2	0.0001
4	0.0008
6	0.0010
8	0.0014
10	0.0018
12	0.0020

The effect of the temperature (40 ^{o}C & 50 ^{o}C) on the stability of solid ciprofloxacin hydrochloride:
Few grams of ciprofloxacin hydrochloride were placed into two Petri dish and placed in ovens thermostated at (40^{o}C & 50 ^{o}C). Samples were taken every month for six months.

25 mg of thermal decomposed ciprofloxacin hydrochloride were transferred to a100 ml volumetric flask, dissolved in distilled -water and the volume completed to 100 ml with the same solvent; 5ml from this solution were pipette and transferred to a 25 ml volumetric flask, diluted to volume with the mobile phase **(Table 4)**.

Table 4: The effect of the temperature (40^{o}C & 50 ^{o}C) on the stability of ciprofloxacin hydrochloride solid

Interval time/ Month	Remaining concentration %	
	40 ^{o}C	50 ^{o}C
1	99.99	99.98
2	99.86	99.77
3	99.74	99.40
4	99.46	99.36
5	99.44	99.35
6	98.49	98.46

The effect of temperature on the reaction rate of ciprofloxacin hydrochloride solution

Ten mls of ciprofloxacin hydrochloride solution (25 mg / 100 ml- water), were pipette and transferred into four 100-ml volumetric flask. The volume of each flask was completed with distilled water. Each volumetric flask was placed into a water-path thermostated at 40, 50, 60, and 70^{o}C separately. Ten ml of each sample were pipette at 30, 60, 90,120 and 150 minute and transferred to stopper tube and cooled in iced water. Five ml from each solution were separately pipette and transferred to 25 ml volumetric flask and the volume completed to the mark with the mobile phase

Table (5): The effect of temperature on the reaction rate of ciprofloxacin hydrochloride solution

Temperature (^{o}C)	Reaction Rate (K)-min^{-1}
30 ^{o}C	0. 00022
40 ^{o}C	0. 00046
50 ^{o}C	0. 00057
60 ^{o}C	0. 00080
70 ^{o}C	0. 00150

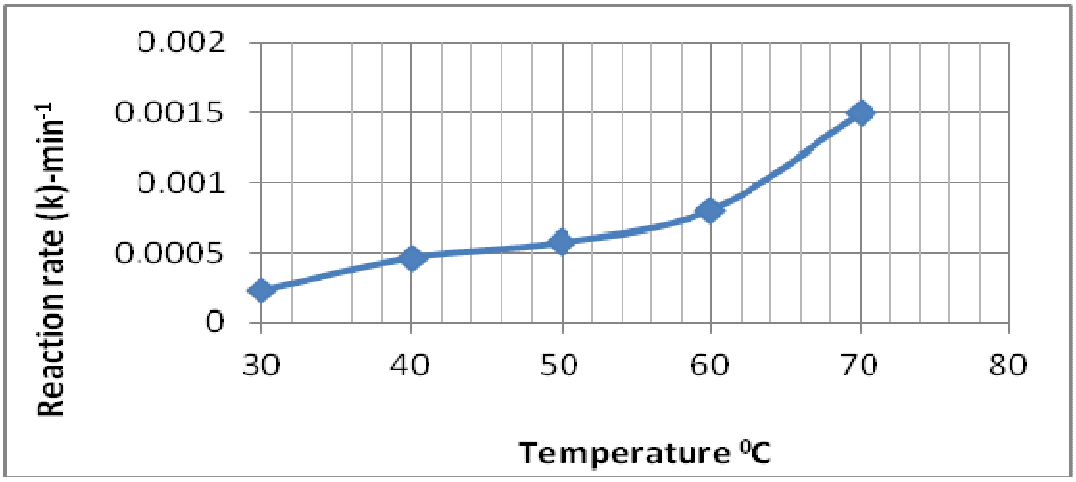

Figure 2. Temperature vs. reaction rate (k) to illustrate the effect of temperature on the reaction rate of ciprofloxacin hydrochloride solution

The effect of pH-value on the reaction rate of Cipro- HCl thermal decomposition

Serial buffer solutions were prepared with pH 2, 4, 6, 8, 10 and 12 according to the method described by Carmody [5].25 ml of ciprofloxacin hydrochloride (25 µg / ml) were pipette, separate transferred into six 100-ml volumetric flask; each flask completed to the volume with one of the universal buffer solutions. Each flask was placed into a water-path thermo stated with shaker at 70^{o}C. 10 ml of samples were taken at 30, 60, 90, 120 and 150 minutes, transferred to stopper tube and cooled in iced water. 5 ml of each solution were pipette, transferred to a volumetric flask 25 ml and diluted to the volume with the mobile **(Table 6)** and **(figure 3)**.

Table (6): The effect of the pH-value on the reaction rate of thermal decomposed of ciprofloxacin hydrochloride

pH-value	Reaction rate (K)-min^{-1}
2	0.0005
4	0.0007
6	0.0013
8	0.0016
10	0.0022
12	0.0034

Effect of pharmaceutical excipients:

The required pharmaceutical excipient was weighted [6], transferred into 100 ml volumetric flask and dissolved with small volume of distilled water. Twenty-five ml of ciprofloxacin hydrochloride (25µg/ml) was added to the flask containing the pharmaceutical excipients; the volume was completed to the mark with distilled water. The flask was exposed directly to sunlight. Samples of 10-mls were taken at 15, 30, 45, 60, 75, 90, and 105 and 120 minutes, each sample was separately transferred into 25ml volumetric flask and diluted to the volume with distilled water and

analyzed by RP-HPLC.The some preparation were repeated in a thermostated water bath (70 ^{o}C) **(Table 7) and (Fig.4)**.

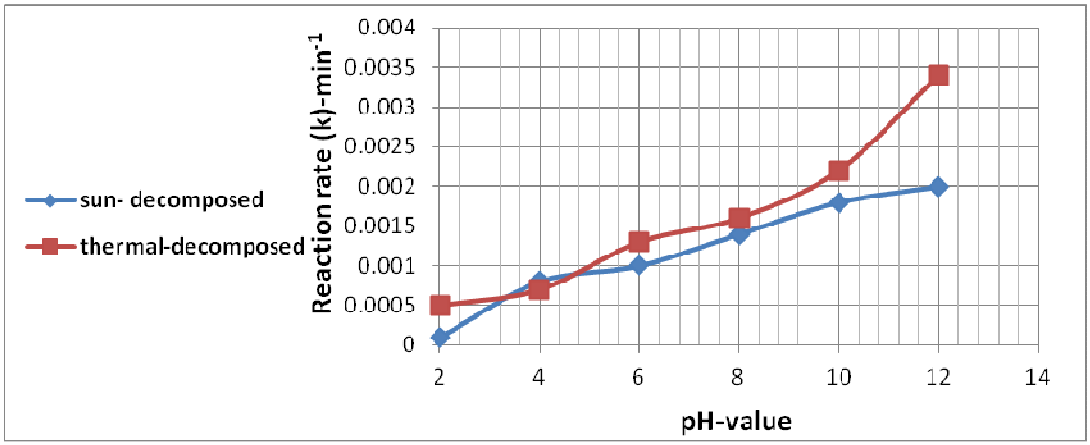

Figure 3. Reaction rate (k) vs.pH-value to illustrate the effect of pH on the reaction rate of ciprofloxacin hydrochloride sunlight and thermal decomposition

Table (7): The effect of some pharmaceutical excipients on the reaction rate of Sun and Thermal-decomposition of ciprofloxacin hydrochloride

Excipient name	Concentration %	pH-value	Reaction rate(K)-min^{-1} Sun decomposed	Reaction rate(K)-min^{1} Thermal decomposed
Methyl paraben	0.1%	(4.60)	0.0015	0.0008
Propyl paraben	0.1%	(5.28)	0.0036	0.0169
Maize starch	0..5%	(5.45)	0.0195	0.0180
Stearic acid	1.0%	(5.74)	0.01703	0.0200
Talcum	1.0%	(6.26)	0.0186	0.0270
MCC	1.0%	(6.73)	0.0186	0.0008
Sodium benzoate	0.05%	(7.17)	0.2703	0.032
Magnesium stearate	0.1%	(7.44)	0.3084	0.0739
Methyl paraben-Na	0.1%	(9.54)	0.2380	0.045
Propyl paraben-Na	0.1%	(9.99)	0.2423	0.0651
Control (no. excipient)	-	(4.30)	0.2395	0.0404

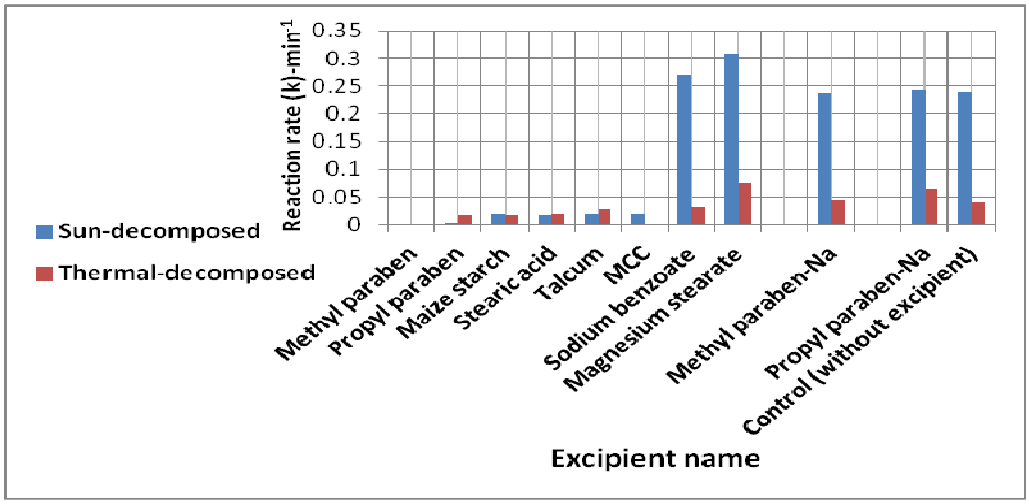

Figure 4. Reaction rate (k) vs. pharmaceutical excipient to illustrate the effect of excipient on the reaction rate of ciprofloxacin hydrochloride sunlight and thermal decomposition.

RESULTS AND DISCUSSION

Literature revealed that, 87 volumes of 0.025M phosphoric acid adjusted with triethylamine to a pH 3.0 ± 0.1 and 13 volumes of a cetonitrile was preferred as it is used as mobile phase used for resolved ciprofloxacin hydrochloride [1].

HPLC method for the analysis of ciprofloxacin hydrochloride using the mobile phase mentioned above with a flow rate 1.5 ml/min, wave length detection at 278 nm on a C18 column (waters 250 × 4.6 mm, 5µm) , revealed good resolution and peak shape for ciprofloxacin hydrochloride .

For quantitative determination of ciprofloxacin hydrochloride, the calibration curve was plotted for the concentration range 2-10µg/ml. Calibration curve plots were constructed using five standard solutions of different concentration .The statistical parameters and linear regression equation calculated from the calibration curve [8].

The linear regression (r^2) was 0.999 **(Fig .1)**.

The stability of the ciprofloxacin hydrochloride solid sun decomposed was tested under sun light for six months; sample was taken every month for test by HPLC. The sun decomposed sample was analyzed by using RP-HPLC which showed one major decomposed product. The results of analyzed sun decomposed ciprofloxacin hydrochloride solid were shown in **(Table 1)**. HPLC method was found satisfactory for the analysis of ciprofloxacin hydrochloride and sun degraded product.

The stability of sun decomposed ciprofloxacin hydrochloride solution was tested by placed sample under sun light; sample was taken every 60 min for test by HPLC. The reaction rate (k) was calculated and found to be increased with increase the time of sample spent under sun light **(Table 2)**.

The photo – thermal stability of Cipro-HCl in aqueous media is found to be pH dependent. The drug was stable in the range of pH 2 to 4 but the maximum instability of Cipro-HCl was attained at pH 12 **(Table 3and 6)** and **(Fig.3).** The thermal stability of ciprofloxacin hydrochloride in its solid state at 40 ^{o} C and 50 ^{o}C for six month were studied; the drug was found to be stable under these conditions **(Table 4)**.

The reaction rate of thermal decomposition of Cipro-HCl at 30,40,50,60, and 70 ^{o} C, increased with the increase in temperature **(Table 5)**. This could be due to the expected increase in the frequency of collisions between the solute and the solvent molecules. These collisions would be of enough activation energy for the molecules to react; this phenomenon can be well explained by Arrhenius equation [7].

Some pharmaceutical excipinents were found to decrease the reaction rate of sun and thermal decomposition of ciprofloxacin hydrochloride in aqueous media following the order of methyl paraben propyl paraben talcum microcrystalline cellulose (M.C.C) and maize starch. Other pharmaceutical excipinents were found to increase the reaction rate of ciprofloxacin hydrochloride sun and thermal decomposition in the following order magnesium stearate sodium benzoate propyl paraben sodium and methyl paraben sodium **(Table 7)** and **(Fig.4)**. It can be observed that pharmaceutical excipients which decrease the reaction rate (K) are acids or esters which may get hydrolyzed to generate the corresponding acid which leads to drop in pH-value. Pharmaceutical excipients which increase reaction rate are alkaline in nature pH-value and therefore increased decomposition **(Table .7)** and **(Fig .4)**. All photos - thermal reactions of ciprofloxacin hydrochloride in aqueous and solid media were found to follow first order reaction kinetics.

CONCLUSION

It can be concluded from this work, that ciprofloxacin hydrochloride is found to be unstable under sun light into solid and liquid forms rather than thermal decomposition. The degradation of ciprofloxacin hydrochloride was more significant in the liquid form than the solid one.

The HPLC method was found to be suitable for the study of kinetic of photo-thermal decomposition of ciprofloxacin hydrochloride and its quantitative determination in the presence of photo-thermal decomposition products.

Some pharmaceutical excipients were found to enhance the stability of ciprofloxacin hydrochloride; therefore it's recommended that such excipients be used in the formulation.

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