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Study of forced degradation of cefexime trihydrate indicating stability using reversed phase high performance liquid chromatographic (RP-HPLC) method

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ABSTRACT

The present study describes the degradation behaviour of cefixime trihydrate which was investigated under different stress conditions viz acidic, basic,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 0.1 N at 80 °C for 30 min and in 0.01 N NaOH at 80 °C for 8 h, the degradation rate was found as >98% and >60% of drug, resp ectively. In acidic condition (0.1 N HCl at 80 °C for 7 h), the degradation was somewhat slower and it was found about 50%.The drug was degraded about 70% and about 30% under exposing UV radiation for 2.5 h.

Keyword: Cefixime, stability, forced degradation, validation

INTRODUCTION

Cefixime ($C_{16}H_{15}N_5O_7S_2$) is a broad spectrum third generation cephalosporin, active against gram positive and gram negative aerobic bacteria [1]. It is clinically used in the treatment of susceptible infections including gonorrhoea, otitis media, pharyngitis, lower respiratory-tract infections such as bronchitis, and urinary-tract infections [2]. Literature survey revealed the estimation of cefixime has been determined alone or along with other drugs by UV [3-5], HPLC [6-9], HPTLC [10-11]. But there are very few reported methods for analysis of degradation product and impurities of cefixime [12-13].

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. It is also recommended that the analyses of stability samples should be carried out by the use of validated stability-indicating testing methods .

This paper deals with the forced degradation of cefixime under stress conditions like acidic hydrolysis, alkaline hydrolysis, oxidation, UV radiation degradation and thermal stress; and also deals with validation of the developed method for the assay of cefixime from its bulk drug and in pharmaceutical dosage forms.

MATERIALS AND METHODS

Drugs and reagents

Working standard of cefixime trihydrate with the potency of 99.29% was a kind gift of Renata Pharmaceuticals Ltd., Dhaka, Bangladesh. Monobasic sodium phosphate buffer (NaH_2PO_4), 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 µm) column was used for the analysis.

Preparation of mobile phase
To prepare buffer solution of pH 6.5, monobasic sodium phosphate (NaH_2PO_4) (195.5 mg) was taken in a 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 6.5 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
11.27 mg of cefixime trihydrate ($C_{16}H_{15}N_5O_7S_2 \cdot 3H_2O$) equivalent to 10 mg cefixime 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 cefixime of 1 mg/mL solution. Further dilution was carried to get concentration of 10, 20, 30, 40, and 50 µg/mL of cefixime.

Sample preparation
Twenty cefixime trihydrate tablets were weighed and the average weight was calculated. Sample equivalent to 10 mg of cefixime 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.

Chromatographic conditions
For quantitative analysis of cefixime by RP-HPLC method, the mobile phase was comprised of monobasic sodium hydrogen phosphate buffer (pH 6.5) and acetonitrile in the ratio of 90:10 (v/v) at a flow rate of 0.7 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 254 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 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 cefixime at three levels of concentrations at three times. The sample solutions were spiked with cefixime 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 cefixime. 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 cefixime trihydrate 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 cefixime
Forced degradation of the drug product was carried out as per the ICH guideline [14]. The forced degradation study of cefixime was performed in acidic, alkaline and oxidant media, under UV and thermal conditions.

Acidic degradation
1 mg/mL of cefixime were prepared by dissolving 56.36 mg of cefixime trihydrate in 50 mL of 0.1N methanolic hydrochloric acid. 25 mL of this solution was refluxed in round bottom flask at 80 ^{o}C in the thermostatically controlled heating chamber. The remaining solution was kept at room temperature. 0.1 mL of the solution was withdrawn at 1st, 3rd, 5th and 8th hour 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 cefixime were prepared by dissolving 56.36 mg of cefixime trihydrate in 50 mL of 0.1N methanolic NaOH and 25 mL of the solution was refluxed in round bottom flask at 80 ^{o}C in the thermostatically controlled heating chamber. The remaining solution was kept at room temperature. 0.1 mL of the solution was withdrawn at 0.5 and 1st hour. Same procedure was carried out in 0.01 N sodium hydroxide. Each time 0.1 mL of the solution was withdrawn at 1st, 3rd, 5th and 8th hour and was diluted with the 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 cefixime solution was exposed to UV radiation at 254 nm. 5 mL of sample solution were taken at 30, 60,120 and 150 minutes. The solutions were diluted with mobile phase. 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 cefixime after forced degradation by using the mobile phase comprising monobasic sodium hydrogen phosphate buffer (pH 6.5) and acetonitrile in the ratio of 90:10 (v/v) at a flow rate of 0.7 mL/min. The injection volume was 20 µL for both standard and samples. The analyses were monitored at 254 nm at ambient temperature. The retention time of cefixime was found to be 8.1 ± 0.1 min by using a C18 column. The specificity of the method was monitored by analyzing the placebo (containing all the ingredients of the formulation except the analyte) (Fig.1).

When average peak areas were plotted against concentration levels of 10, 20, 30, 40 and 50 µg/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 of the newly developed method (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 100.06%, 99.97% 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 0.73 – 1.26% 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 0.016 µg/mL and 0.16 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 cefixime. Therefore, the method was applied for quantitative analysis of forced degraded products of cefixime.

In the basic degradation study, it was found that cefixime was more sensitive to alkaline hydrolysis. More than 98% of drug was degraded in 30 min if heated with 0.1N sodium hydroxide at 80 ^{o}C. In 0.01N sodium hydroxide it was somewhat stable and only approximately 60% of drug was degraded in 8 h at 80 ^{o}C (Fig. 4). The degradation was somewhat slower in acidic conditions and it was about 50% when heated with 0.1N hydrochloric acid at 80 ^{o}C for 7 h (Fig. 5).

After exposing of cefixime trihydrate to UV radiation for 150 min at 254 nm, the drug content was decreased to about 70% (Fig. 6).

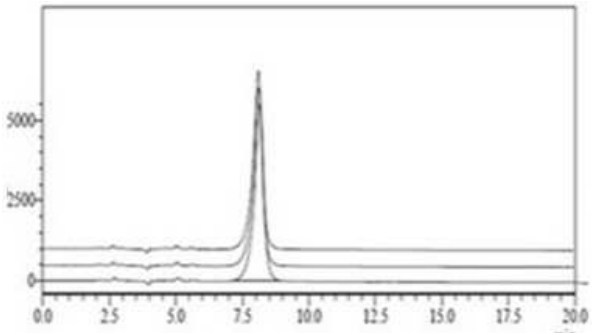

Fig. 1 System Suitability

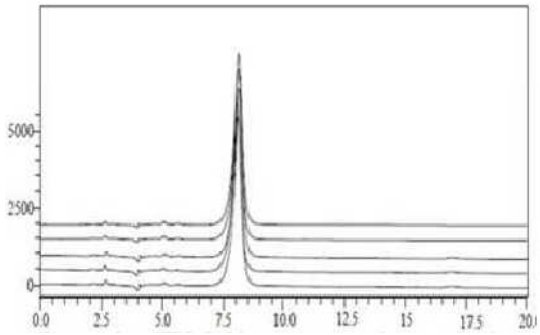

Fig.2 HPLC Chromatogram for Calibration curve

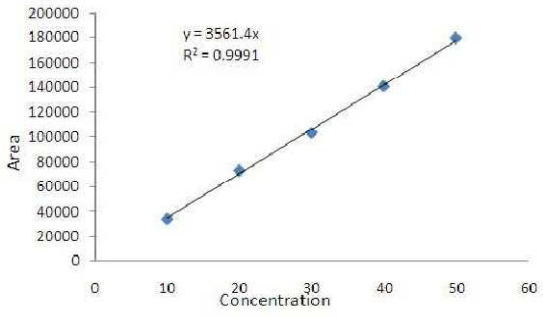

Fig. 3 Calibration curve of cefixime

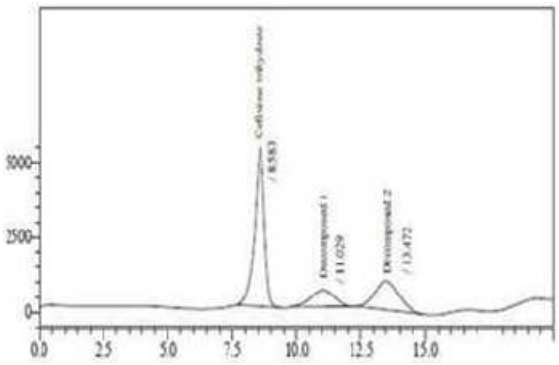

Fig.4 Alkaline degradation

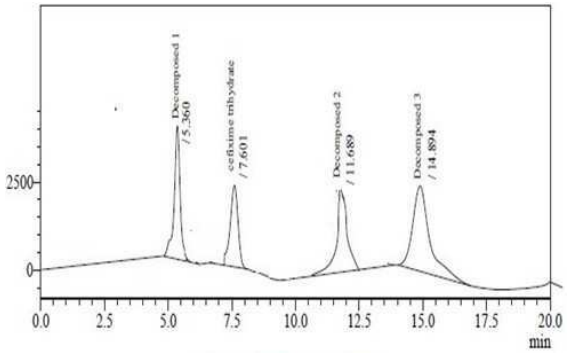

Fig. 5 Acidic degradation

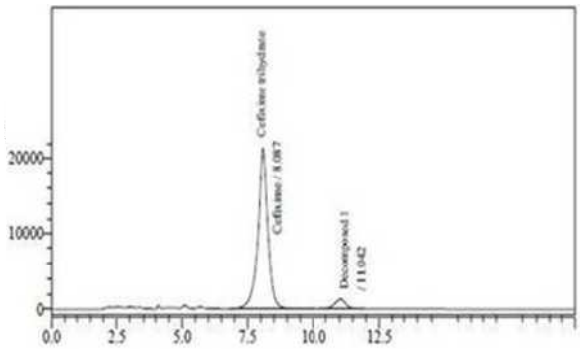

Fig.6 UV degradation

Table 1 Accuracy of cefixime.

Run	Injected %concentration level	Recovered % concentration level	% Recovery	%Mean
1		50.14	100.28	
2	50	49.97	99.94	100.06
3		49.98	99.96	
1	100	100.11	100.11	
2		99.78	99.78	99.97
3		100.01	100.01	
1	150	150.13	100.09	
2		149.95	99.97	100.02
3		149.99	99.99	

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

Inter-day precision			
Concentration (µg/mL)	Mean Area	SD	% RSD
20	73706	1021	1.39
40	141272	1523	1.08
60	213545	2765	1.29
Intra-day precision			
20	77512	1045	1.35
40	152468	2015	1.32
60	209875	2421	1.15

Table 3 Result for robustness study

Change in flow rate	Retention time	Mean	% RSD
0.8	8.7	120457	0.73
1.0	8.3	141272	1.13
1.2	7.9	184562	1.17
Change in organic composition in the mobile phase			
5% less	8.1	112436	0.81
Actual	8.3	141272	1.26
5% more	8.2	219031	1.17

CONCLUSION

Forced degradation of cefixime 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 in alkaline condition, drastic degradation occurred. In acidic condition the degradation was somewhat slower. The drug was also degraded under exposing UV radiation.

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