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Study of degradation of cefixime trihydrate under stress conditions using stability indicating reverse phase -high performance liquid chromatography method

Elsadig H. K. Adam [1], Ahmed E. M. Saeed *[2], Izzeldin E. Barakat [3]

[1]*Amipharma Laboratories Ltd, Khartoum, Sudan*
[2, 3] *Department of Chemistry, Collage of Science*, Sudan University of Science and Technology

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ABSTRACT

The present study describes degradation behavior of cefixime trihydrate (CEF-3H_2O) which was investigated under different stress conditions of sun light, UV light at 254 nm and some pharmaceutical excipients using HPLC. Stability - indicating methods RP-HPLC 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 UV light, two major decomposed products were successfully resolved on a C18 column (waters spherisorb 25 cm × 4.6 mm,5µm),utilizing mobile phase of tetra butyl ammonium hydroxide solution (0.03M aqueous) pH adjusted to 6.5 with diluted orthophospharic acid (10 % aqueous) and acetonitrile in a ratio of 3:1 respectively. Mobile phase was delivered at the flow rate of 1.0 ml/min. Ultra violet detection was carried out at 254 nm. Separation was completed within 4.62, 11.05 minutes respectively. 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.TLC technique was used to separate two decomposed products which were appeared at (R_f) 0.65, 0.78 respectively for UV decomposed and 0.39, 0.65 respectively for sun decomposed. Some pharmaceutical excipinents were found to decrease the reaction rate of sun, UV, and thermal decomposition of cefixime trihydrate in aqueous media. Other pharmaceutical excipinents were found to increase the reaction rate of cefixime trihydrate sun, UV, and thermal decomposition.

Keyword: Cefixime trihydrate, Stability, UV light degradation, Sunlight degradation, pharmaceutical excipients.

INTRODUCTION

Cefixime is a semi synthetic, aminothiazolyl, broad spectrum third generation cephalosporin, active against gram positive and gram negative aerobic bacteria. The chemical formula of cefixime trihydrate is $C_{16}H_{15}N_5O_7S_2.3H_2O$**,** molecular weight 507.50 as trihydrate. Chemically, it is (6R, 7R)-7-{[(Z)-2-(2-aminothiazole-4-yl)-2- [(carboxymethoxy) imino] acetyl] amino]-3-ethyenyl-8-oxo-5-thia-1-azabcyclo [4.2.0] oct-2-ene-2-carboxylic acid trihydrate [1], [2].

Degradation reaction in pharmaceutical formulations take place at definite rates and are chemical in nature [3].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 [4].
These studies help to determine the significant related substances to be used in method development [5], and to determine the degraded product formed under Sun light and UV light stress conditions, and the effect of pharmaceutical excipients on the reaction rate of cefixime trihydrate [6].

The present study aimed at investigation of the photo-thermal stability of cefixime trihydrate.

MATERIALS AND METHODS

Chemicals and Reagents:

All chemicals and reagents used were of a HPLC grade .Cefixime trihydrate was kindly supplied from AUROBINNDO PHARMA LTD –INDIA. Tetra-n-butyl ammonium hydroxide 40% aqueous solution was obtained from AppliChem, Germany. Acetonitrile HPLC grade was obtained from BDH Labs, England.Orthophosphoric acid 85 % was obtained from BDH laboratory, England.

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, Waters Spherisorb®5.0µm ODS2 4.6 mm x 250mm.
3. Sartorius model cp224s balance
4. Mi 180 Bench pH meter, MARTINI instruments.
5. Ultra violet radiation (UV Lamp λ254 - λ 265), Obligatory eye protection
-Model-M014492 BDH –England

Preparation of 0.03 M tetra butyl ammonium hydroxide solution

Solution was prepared by diluted 20 ml of tetra butyl ammonium hydroxide 40 % aqueous solution to 800 ml with distilled water and adjusted to pH 6.5 with 10% orthophosphoric acid and diluted up to 1000 ml with distilled water.

The mobile phase was prepared by mix a solution of 0.03 M tetra butyl ammonium hydroxide and acetonitrile with a ratio of 3:1 respectively and degassed [7].

The stock standard solution having concentration of 0.1 mg/ml was prepared by dissolving pure drug of cefixime trihydrate in distilled water.

The calibration curve solutions were obtained by further diluted stock solution to get concentrations of 10, 20, 30, 40, and 50µg/ml of cefixime trihydrate.

Preparation of cefixime trihydrate UV decomposed product:

100 µg / ml of cefixime trihydrate aliquots were prepared and transferred to a stoppered tube. The solutions were placed under UV radiation at λ 254 nm. Five ml of samples were taken at 30, 60, 90, 120, and 150minutes, transferred to 25ml volumetric flask and the volume was completed to the mark with distilled water and analyzed by RP-HPLC.

The aliquots of cefixime trihydrate UV decomposed extracted by 3x 40 ml ethyl acetate, the ethyl acetate layers collected into flask round bottom 250 ml and evaporated by using rotary evaporator until to dryness. The extract was dissolved in methanol, and TLC separated by silica gel $60F_{254}$ in a mobile phase of methanol: chloroform: concentrate ammonium with a ratio 10:10:0.15 respectively.

Preparation of solid sample for Sun decomposition

Few grams of cefixime trihydrate 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 2010). Samples were taken every month and tested for degradation by RP-HPLC.100 µg / ml of cefixime trihydrate sun decomposed 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 cefixime trihydrate were calculated.

Cefixime trihydrate sun decomposed was TLC separated by silica gel $60F_{254}$. In a mobile phase of methanol: chloroform: conc ammonia with a ratio 10:10:0.15 respectively.

The effect of time on the stability of cefixime trihydrate solution

100 µg/ml water of CEF-$3H_2O$ was prepared and directly exposed to sunlight. Samples were taken at interval time 0, 30, 60, 90, 120 and 150 minutes.

Ten ml from the solution was transferred to stoppered tubes and cooled with iced water; five ml from each solution were pipetted, transferred separately to 25 ml volumetric flask and diluted to volume with distilled water and analyzed by RP-HPLC

Effect of pharmaceutical excipients:

The required pharmaceutical excipient was weighted [8], transferred into 100 ml volumetric flask and dissolved with small volume of distilled water. Twenty-five ml of cefixime trihydrate (10 mg /100 ml-water) 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 under UV light and in a thermostated water path (70 ^{o}C).

RESULTS AND DISCUSSION

Literature revealed that, tetra butyl ammonium hydroxide solution mix with acetontrile with a ratio of 3:1 was preferred as it is used as mobile phase used for resolved cefixime trihydrate.

HPLC method for the analysis of cefixime trihydrate using the mobile phase above mentioned above with a flow rate 1.0 ml/min, wave length detection at 254 nm on a C18 column (waters 250 × 4.6 mm, 5µm) , revealed good resolution and peak shape for cefixime trihydrate (Figure .2).

For quantitative determination of cefixime trihydrate, the calibration curve was plotted for the concentration range 10-50µg/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 is given in table (2) .The linear regression (r^2) was 0.999 (Figure .1). Limit of Detection (LOD) was calculated by using formula 3.3(σ/S) where σ is standard deviation of the response and S is slope of the calibration curve. LOD was found to be 0.41 µg/ml. Limit of Quantitation (LOQ) was calculated by using formula 10(σ/S) was found to be 1.24 µg/ml.

The effect of UV light on the stability of cefixime trihydrate solution

With the above selected method parameters (Table.1), system suitability testing provided adequate good resolution for analysis to be performed (Table.3), the resolution of cefixime trihydrate and its degradation products are shown in figure (3), two major decomposed products were obtained .The stability of the cefixime trihydrate solution was tested under UV light at 254 nm for different interval times. The UV sample was analyzed using RP-HPLC. The results of UV decomposed products were shown in table (4) and figure (4). HPLC method used was found to be satisfactory for analysis of cefixime trihydrate and degraded products. System suitability parameters were used for the analysis of cefixime trihydrate UV decomposed, results obtained were given in table (3). Different concentrations in the range of 10, 20 and 30 µg/ml were used to determine the effect of concentration on the reaction rate of cefixime trihydrate UV decomposed .The results obtained revealed that with a decreased concentration there was increased reaction rate (Table .5 and Figure .5).

Solvents of methanol and chloroform in different ratios and strong ammonia were used for the separation of UV decomposed products by silica gel $60F_{254}$ plate .Mobile phase of methanol: chloroform: strong ammonia solution 30% (10:10:0.15) was found to be satisfactory for separation of UV decomposed products. Two major decomposed products at relative distance (R_f) of 0.75, 0.65 respectively were isolated (Figure .6) which were found to be typical to decomposed (1) and decomposed (2) respectively which were appeared in figure (3). The two major decomposed products were isolated.

The effect of sun light on the stability of cefixime trihydrate solid

The stability of the cefixime trihydrate 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 products were shown in table (7) and figure (6, 7). HPLC method was found satisfactory for the analysis of cefixime trihydrate and sun degraded product.

The effect of sun light on the stability of cefixime trihydrate solution

The stability of the cefixime trihydrate solution sun decomposed was tested by placed sample under sun light for 150 minutes; sample was taken every 30 min 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 products were shown in table (8) and figure (8). The reaction rate (k) was calculated and found to be increased with increase the time which sample spent under sun light.

The effect of some pharmaceutical excipients on the reaction rate of cefixime trihydrate sun decomposition

Some pharmaceutical excipinents were found to decrease the reaction rate of sun, UV and thermal decomposition of cefixime trihydrate in aqueous media following the order of monobasic sodium phosphate (monobasicNa-ph), EDTA, and magnesium stearate (Mg.stearate). Other pharmaceutical excipinents were found to increase the reaction rate of cefixime trihydrate

sun, UV and thermal decomposition in the following order sodium citrate dibasic (NaC-dibasic), sodium citrate tribasic (NaC-tribasic), dibasic sodium phosphate (DibasicNa-ph), and Talcum. 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 which increase pH-value and therefore increased decomposition (Table .9) and (Figure .9).

All the photo (Sun light and UV light) reactions of cefixime trihydrate in aqueous and solid media were found to follow first order reaction kinetics.

Table 1. Conditions used for chromatography analysis

Sr .NO	Parameter	Condition used for analysis
1	Mobile phase	Tetra butyl ammonium hydroxide solution (0.03 M)adjusted to pH 6.5 with 10 % orthophosphoric acid: Acetonitrile (3:1)
2	Flow rate	1.0 ml/min
3	Detection Wavelength	254 nm
4	Sample injector	50µl loop
5	Column	C18 waters (4.6 x 250 mm,5µm)

Table 2. Results of standard calibration curve for the analysis of cefixime trihydrate by HPLC (r^2 = 0.99985)

Concentration of cefixime trihydrate µg/ml	peak height
10	13207.32
20	28836.83
30	42563.71
40	57307.51
50	72157.81

LOD = 0.41 µg/ml
LOQ = 1.24 µg/ml

Table 3. System Suitability testing for cefixime trihydrate solution UV decomposed

Component	Retention time(min)	No. of Total Plates	Resolution	Asymmetry 5 %
Decompose 1	4.62	3389	8.974	0.95
Decomposed 2	11.049	5000	3.294	0.97
Remaining CEF-3H_2O	13.03	4925	0.000	1.05

Table 4. Stability analysis of cefixime trihydrate solution UV decomposed

Interval time/min	Remaining CEF-3H_2O % R.T 13.0 ± 0.5min	Decomposed(1)% R.T 4.62 ± 0.5min	Decomposed(2)% R.T11.049 ±0.5min	Reaction rate (K)-min^{-1}
30	72.84	5.21	11.90	0.0104
60	58.12	9.40	18.72	0.0194
90	49.38	12.49	21.57	0.0272
120	36.01	16.41	22.59	0.0357
150	29.54	17.64	21.74	0.0438

Table 5. Effect of different concentration of cefixime trihydrate in UV decomposition

Conc. µg/ml	%Remaining of CEF-3H_2O	%Composition of decomposed 1	%Composition of decomposed 2	Reaction rate K-min-1
10	29.54	17.64	25.74	0.0081
20	55.22	13.29	21.09	0.0039
30	68.22	5.11	14.55	0.0025

Table 6. Isolation and characterization of the two major decomposed products of cefixime trihydrate aliquots UV decomposed results

Peak No. into HPLC Chromatogram	R.T. min	Type of Peak	Spot No. into TLC Plate	R_f	Type of Spot
1	4.6	decomposed 1	1	0.56	CEF-3H_2O
2	11.1	decomposed 2	2	0.65	decomposed 2
3	13.0	CEF-3H_2o	3	0.78	decomposed 1

Tablet 7. Analysis of cefixime trihydrate solid sun decomposed by HPLC

Interval time/month	%Remaining CEF-3 H_2O R.T 13.0 ± 0.5min	% Decomposed R.T 5.90 ± 0.5min	Reaction rate(K)-month^{-1}
1 month	89.31	2.11	0.0960
2 months	86.71	5.78	0.1589
3 months	61.42	14.77	0.3158
4 months	36.31	24.94	0.5649
5 months	20.07	36.46	0.8828
6 months	13.01	44.08	1.2199

Table 8. The effect of time on the stability of cefixime trihydrate solution sun-decomposition

Inter val time /min	Decomposed %	Remaining CEF-3 H_2O %	Reaction rate (K)- min $^{-1}$
30	4.72	84.34	0.0051
60	7.43	73.03	0.0101
90	9.22	63.15	0.0150
120	10.01	51.26	0.0204
150	10.24	44.09	0.0257

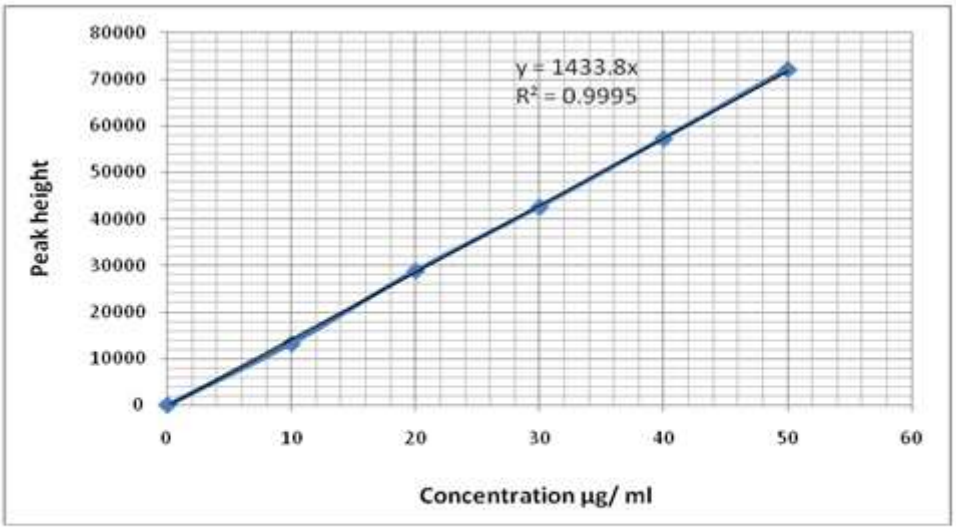

Figure 1. Concentration vs. peak height of standard solution to illustrate the calibration curve for the analysis of cefixime trihydrate by HPLC (r^2 = 0.9995)

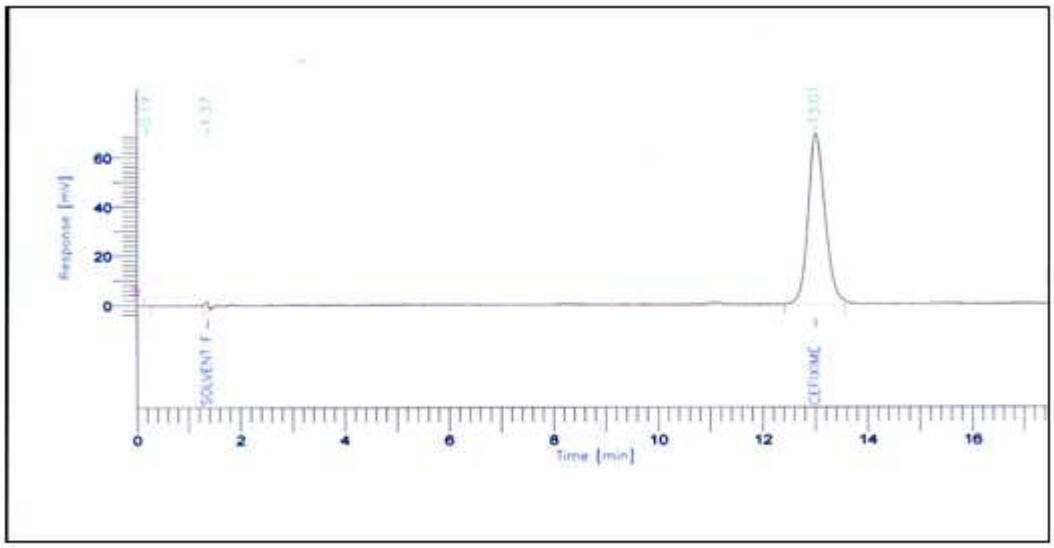

Figure 2. HPLC chromatogram for the analysis of cefixime trihydrate reference standard

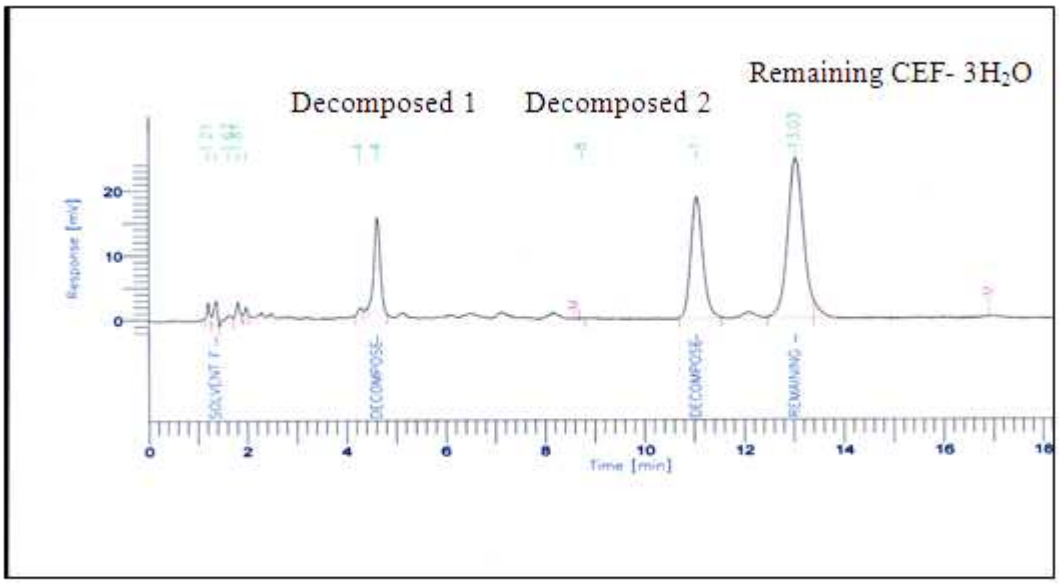

Figure 3. HPLC chromatogram for the analysis of cefixime trihydrate solution UV decomposed to illustrate resolution study

Table 9. Effect of some pharmaceutical excipients on the reaction rate of CEF-3H_2O sunlight, UV light, and thermal decomposition

Excipient name	Concentration %w/w	pH	Reaction rate (k)- min^{-1}		
			Sun	UV	At 70 ^oC
NaC- Dibasic	0.05%	6.95	0.0025	0.0013	0.0005
Monobasic Na-ph	0.05%	6.95	0.0015	0.0009	0.0010
EDTA	0.05%	7.02	0.0016	0.0021	0.0007
Mg-Stearate	0.01%	7.29	0.0014	0.0025	0.0001
Talc	0.01%	7.29	0.0042	0.0039	0.0011
Na C -Tribasic	0.05%	7.32	0.0025	0.0013	0.0018
Dibasic Na-ph	0.05%	7.65	0.0023	0.0014	0.0020
Control (CEF-3H_2O without excipients)	-	6.85	0.0034	0.0012	0.0014

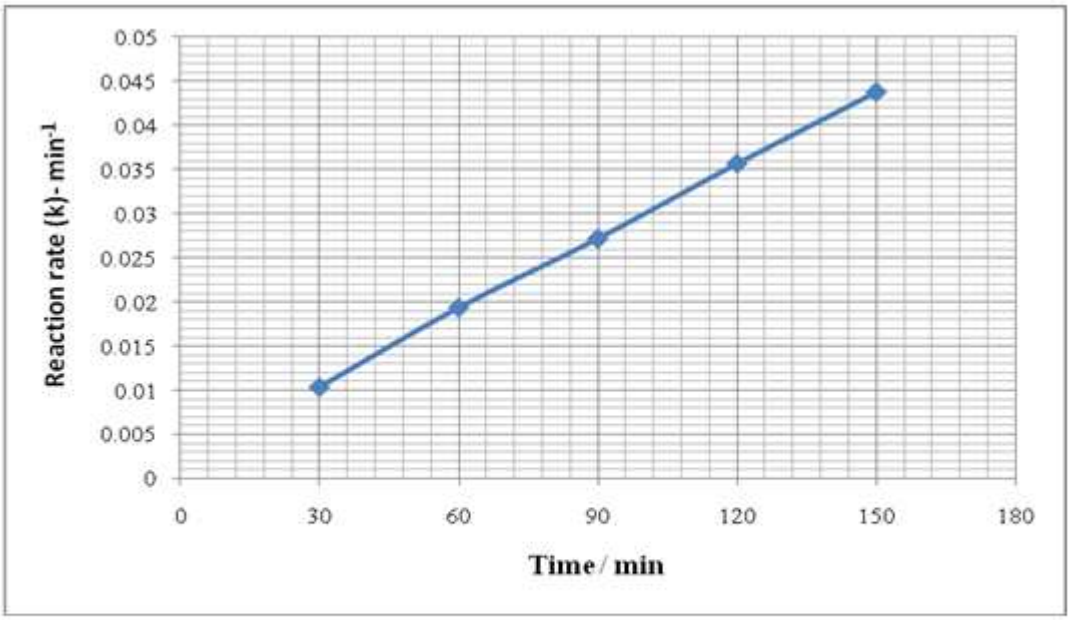

Figure 4. Time vs. reaction rate (K) for the effect of UV light (□ 254 nm) on the stability of cefixime trihydrate solution.

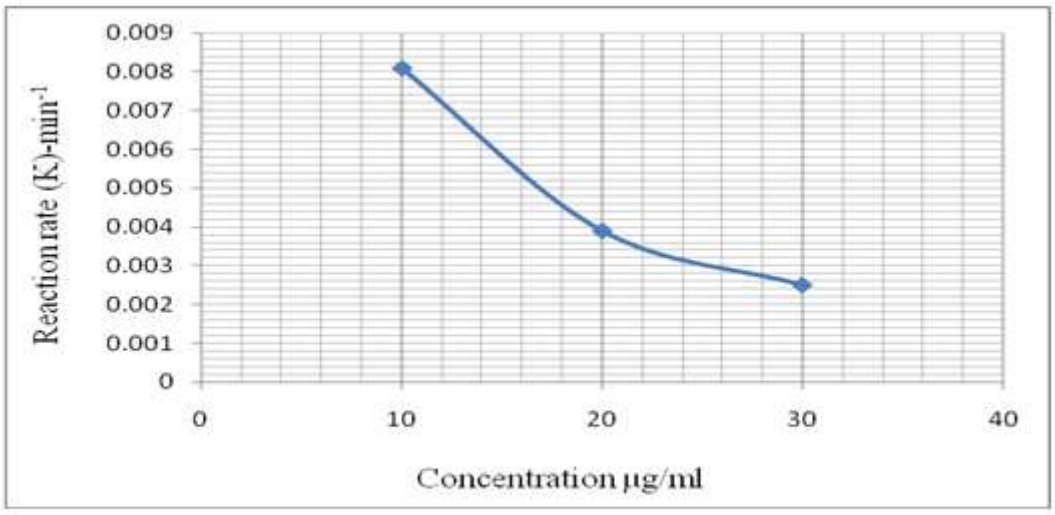

Figure 5. Concentrations vs. reaction rate (K) to illustrate the effect of concentration on the reaction rate of cefixime trihydrate solution UV decomposed

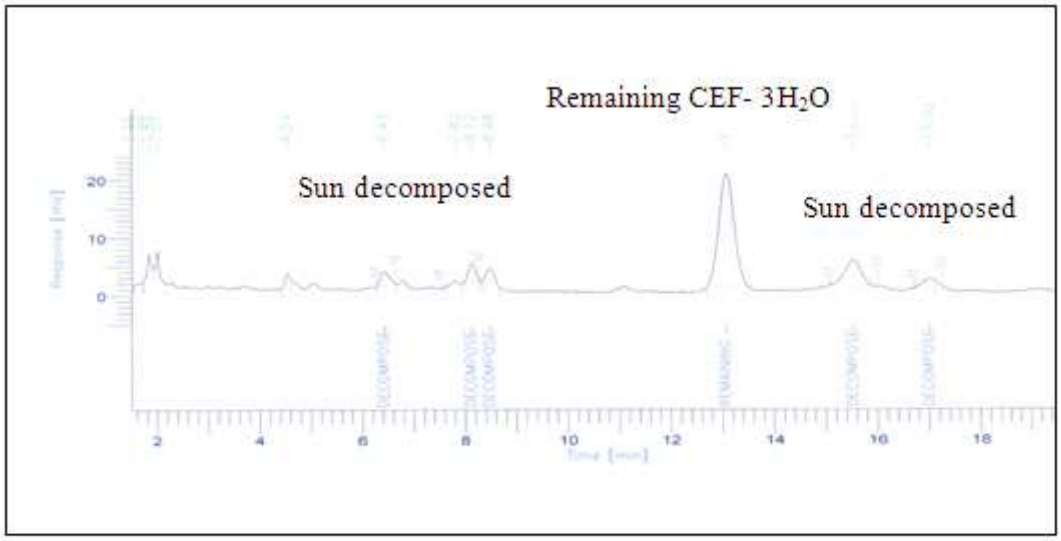

Figure 6. HPLC chromatogram for the analysis of cefixime trihydrate solid sun decomposed after six months.

Figure 7. Time vs. reaction rate (K) for the effect of sun Light on the stability of cefixime trihydrate solid

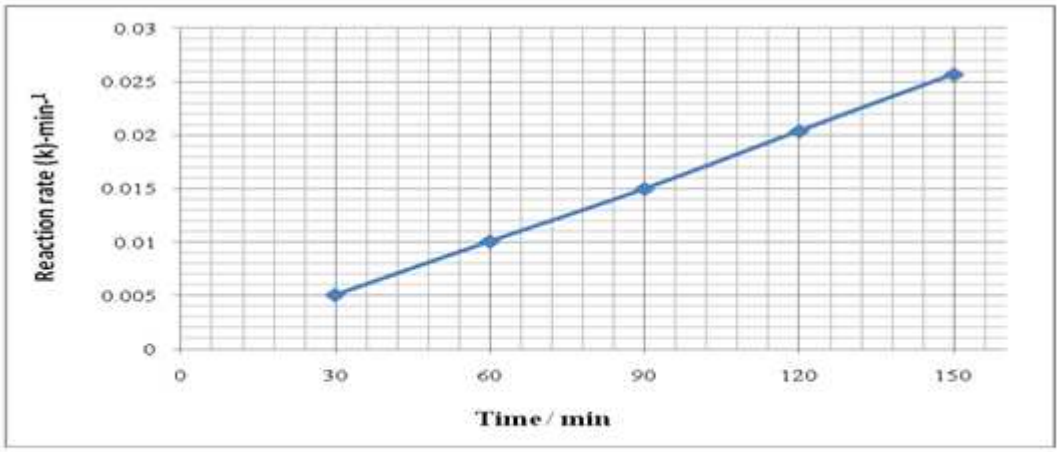

Figure 8. Times vs. reaction rate (K) illustrate the effect of time on the stability of cefixime trihydrate solution sun-decomposed

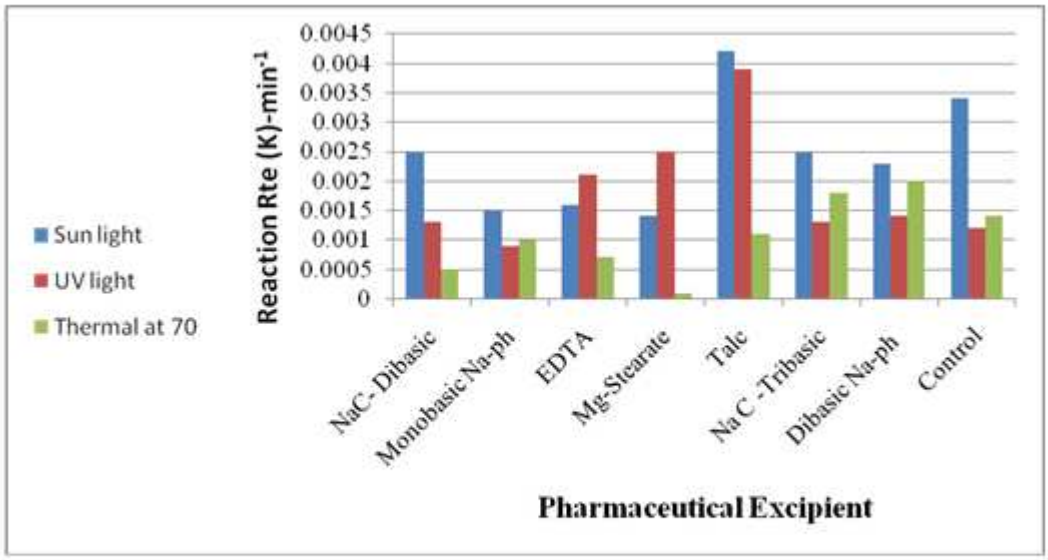

Figure 9. Excipients vs. reaction rate (K) for the effect of sun Light, UV light and thermal at 70 ^{0}C on the stability of cefixime trihydrate

CONCLUSION

It can be concluded from this work, that cefixime trihydrate is found to be unstable under sun light and UV light into solid and liquid forms. The degradation of cefixime trihydrate 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 cefixime trihydrate and its quantitative determination in the presence of photo-thermal decomposition products.

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

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