Degradation study of different brands of Ceftriaxone injection available in Aden city

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Abstract

Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the drug molecules.

Five different brands of Ceftriaxone injection were collected from the market and exposed to various stress condition like alkaline, acidic, oxidative and thermal degradation .The degradation process was followed by using spectrophotometric analysis.

All the antibiotic brands undergo forced degradation at conditions in different ranges and the maximum degradation observed in the basic medium.

Keywords: forced degradation**,** Ceftriaxone injection, Aden city.

Introduction:

Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule. There are three types of stability studies which are long term stability studies, accelerated stability studies and intermediate testing[8,23,6,11]**.**
Stability testing provides information about degradation mechanisms, potential degradation products, possible degradation pathways of drugs, as well as interaction between drugs and excipients in pharmaceuticals. Results are applied in developing a suitable manufacturing process, selecting proper packaging, storage conditions, product's shelf life and expiration dates[22,5,21,13,7,24].
Ceftriaxone for injection is the 3rd degeneration cephalosporin beta-lactam antibiotic used in the treatment of infections due to susceptible Gram-positive and Gram-negative bacteria, including infections of the abdomen, bones and joints, central nervous system, skin and skin structure, genito-urinary tract (including gonorrhea) and respiratory tract, in gynecological infections, and in early Lyme disease[15].

The bactericidal activity of Ceftriaxone results from the inhibition of the cell wall synthesis and is mediated by Ceftriaxone binding to penicillin-binding proteins. It inhibits the mucopeptide synthesis in the bacterial cell wall. The beta lactam moiety of Ceftriaxone binds to carboxypeptidase, endopeptidase and transpeptidase in the bacterial cytoplasmic membrane. These enzymes are involved in cell wall synthesis and cell division. By binding to these enzymes, Ceftriaxone results in the formation of defective cell walls and cell death.

The applications of colorimetric reagents are not a new technique and Prussian blue complex method is a simple, sensitive and accurate spectrophotometric method for the analysis of ceftriaxone, cefotaxime and cefuroxime in pharmaceutical dosage forms and has been developed and validated. The method is based on the formation of Prussian Blue (PB) complex. The reaction between the acidic hydrolysis product of the antibiotics with the mixture of Fe^{3+} and hexacyanoferate (III) ions was evaluated for the spectrophotometric determination of the antibiotics. The maximum absorbance of the coloured complex occurred at $\lambda = 700$ nm[16.17,3]**.**
Literature survey describes that there are many degradation studies of ceftrixone in various stress conditions like alkaline, acidic, oxidative, thermal and photo degradation by using spectrophotometric[4,9,10,18],microbiological[14&1] and HPLC methods .[20,2,12,18] So this paper deals with the forced degradation of ceftrixone under stress conditions like acidic hydrolysis, alkaline

hydrolysis, oxidation and thermal stress and to our knowledge this research is the first work performed with the purpose to compare the degree of forced degradation in five different brands of Ceftriaxone sodium injection (500mg) available in Aden market.
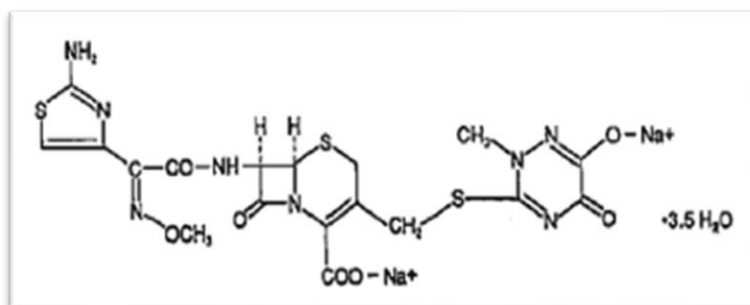

Fig.1. Chemical Structure Of Ceftriaxone Sodium.

Materials and Methods
Materials and instruments:
Sodium hydroxid , Hydrogen peroxide and Ferric chloride(LOBA CHEMIE) , Hydrochloric acid (sigma Aldrich), potassium hexacyanoferrate(III) (Labtch chemicals) .
A water-bath thermo stated (heater and shaker) lab. Tech . model LSB0305, Spectrophotometer unico 2100.
Five different brands of ceftriaxone 500mg injection were purchased from local Aden market (Table 1 shows the manufacturing and expiry date of different brands),and the experimental work was done in College of Pharmacy, during July – September 2016 .

Table(1): Manufacturing and expire date of five brands

Brand code	Mfg. Date	Exp. Date
CTX 1	**Nov-14**	**Nov-17**
CTX 2	**Jan-16**	**Jan-19**
CTX 3	**Apr-16**	**Sep-19**
CTX 4	**May-14**	**May-17**
CTX 5	**Feb-19**	**Feb-19**

Methods:
Preparation of Standard Stock Solution of Ceftriaxone Drug (5mg/ml)
The solution of ceftriaxone was prepared by taking 500mg of ceftriaxone injection dissolved in 5ml of sterile water and transferred into 100ml conical flask and the volume was made up to the mark with de-ionized water.
Preparation of working Solution of Ceftriaxone Drug (1mg/ml)
50ml of Standard Stock Solution of Ceftriaxone (5mg/ml) was withdrawn from the above prepared solution and diluted to 250ml by de-ionized water .
Estimation of ceftriaxone concentrations
The ceftriaxone concentration before and after degradation was estimated by measuring Prussian Blue Formation by taking 1ml from drug solution under study in separated small flask , then 1ml of 0.02M $FeCl_3$ and 0.5ml of potassium hexacyanide was added to drug solution and the volume was made up to 10ml with de-ionized water. Absorbance of the colored complex solution was recorded by spectrophotometer at the wavelength 700nm.

Studying the effect of acidic medium
    10ml from the working solution of each brand was taken in small conical flasks then 10ml of 0.1M HCl was added in each conical flask and , after one hour ,the concentration of antibiotic was estimated.
Studying the effect of basic medium
    10ml from the working solution of each brand was taken in small conical flasks, then 10ml of 0.1M NaOH was added in each conical flask and , after one hour, the concentration of antibiotic was estimated.
Studying the effect of oxidation
   10ml the from working solution of each brand was taken in small conical flasks then 10ml of 3% H_2O_2 was added in each conical flask and , after one hour , the concentration of antibiotic was estimated.
Studying the effect of heat
    10ml from the working solution of each brand was taken in separated small conical flasks. then the solution was put in water bath 60°C for a period of 1 hour. The study of the kinetic degradation was carried out by taking 10ml from the working solution of each brand was taken in Pyrex volumetric flask , then these solutions were placed in water bath for 2 hours at 60°C , and every 30 minutes 1ml of the antibiotic solution was withdrawn in separated test tube for further treatment to calculate the rate of degradation.

Results and discussion
    The table (2) the results of ceftriaxone forced degradation indicates the effects of acidic medium ,basic medium and oxidative medium .The table shows the variation in absorbance before and after the effect of different degradation parameters after one hour exposure.

Table (2): Absorbance of drugs in different parameters after one hours.

Brand name	Absorbance of initial solution	Absorbance after acid effect	Absorbance after base effect	Absorbance after oxidation effect
CTX 1	**1.339**	**1.207**	**0.251**	**0.637**
CTX 2	**1.386**	**1.046**	**0.261**	**1.104**
CTX 3	**1.194**	**0.78**	**0.449**	**0.743**
CTX 4	**1.670**	**0.869**	**0.514**	**1.361**
CTX 5	**1.334**	**0.805**	**0.577**	**0.87**

Acidic medium effect
The previous research (9) indicated that the fluoroquinolones degradation has happened due to the stress acidic by decarboxylation process. The maximum degradation for acidic pH effect was found in CTX4 and CTX3 which is equal to 47.96% and 43.67% respectively, but the minimum degradation was 9.68%   , as shown by CTX1(Fig.2).
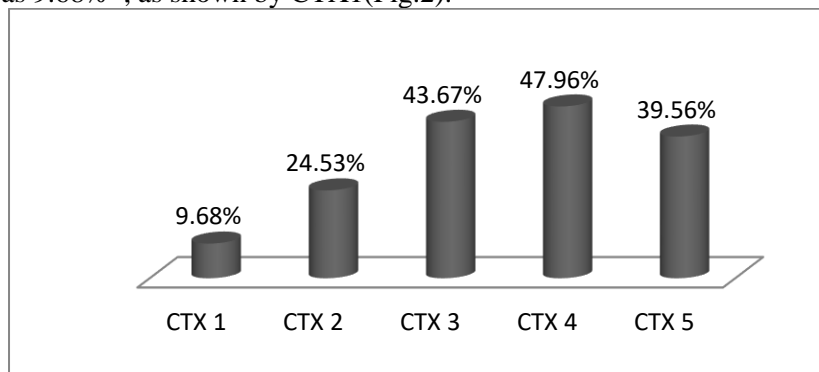

Fig.2: Effect of acidic pH

Basic medium effect

The alkaline degradation of the ceftriaxone, yielded to the opening of the main active bactericidal part of the antibiotic, the beta lactam ring [9].The alkaline degradation of the ceftriaxone, yielded to the opening of the main active bactericidal part of the antibiotic, the beta lactam ring [9].It was observed that the degree of the degradation due to the basic pH effect was found very high comparatively with acidic pH effect, the maximum percent revealed in CTX1 (81.25 %) and CTX2 (81.17%) , but the lowest value was 56.75% for CTX5 (Fig.3).
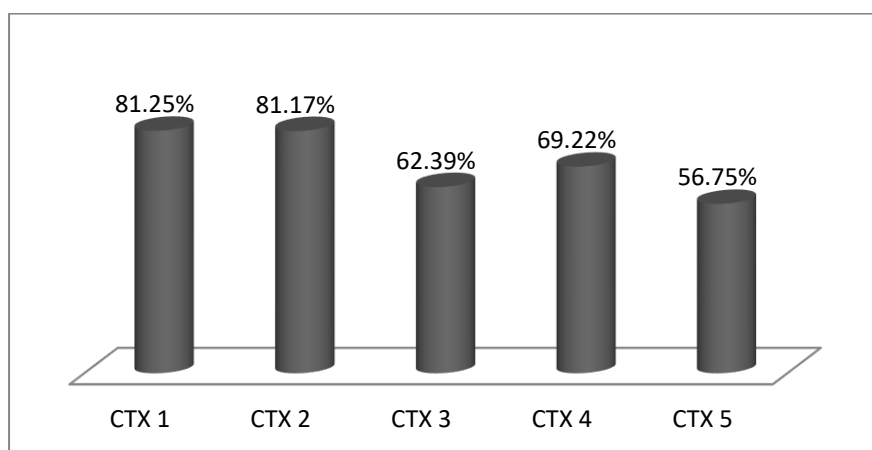

Fig.3: Effect of basic pH

Oxidation effect

The oxidative degradation of drug substance involves an electron transfer mechanism to form reactive anions and cations. Amines, sulfides and phenols are susceptible to electron transfer oxidation to give N-oxides, hydroxylamine, sulfones and sulfoxide. The functional group with labile hydrogen , like benzylic carbon, allylic carbon, and tertiary carbon or α-positions with respect to heteroatom , is susceptible to oxidation to form hydroperoxides, hydroxide or ketone [8].
In case of the Ceftriaxone injection oxidation study by hydrogen peroxide, the degradation degree was found low ,it was 18.5% and 20.35% for CTX4 and CTX2 respectively , but the highest degradation registered in CTX1 brand was 52.43 % (Fig.4).
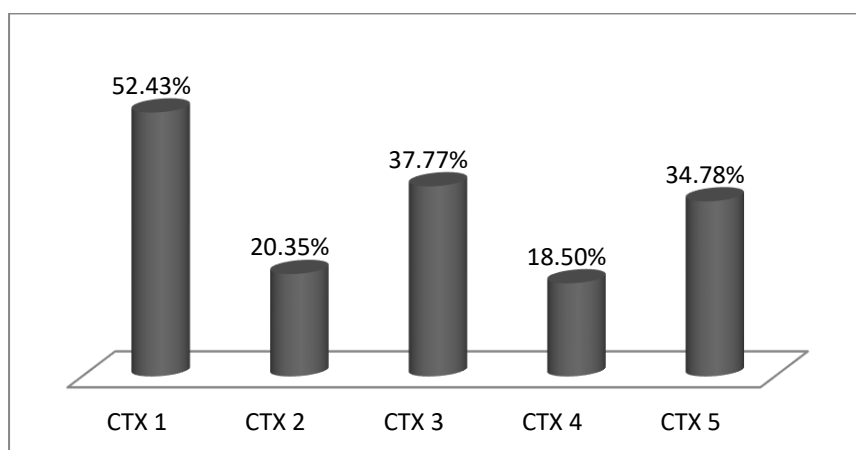

Fig.4: Effect of oxidation

Heat effect :

The thermal degradation results (table 3) of different brands of ceftriaxone indicated that the absorbance decreased in all the brands and the maximum degradation was registered in CTX5 (67.47%) than the other antibiotic brands which resist the thermal degradation.

Table (3): Absorbance of drug before and after heat effect at 60 C°

Brands code	Absorbance of initial solution	Absorbance after heat
CTX 1	**1.339**	**0.996**
CTX 2	**1.386**	**1.023**
CTX 3	**1.194**	**0.815**
CTX 4	**1.67**	**0.916**
CTX 5	**1.334**	**0.434**

Conclusion:

All the antibiotic brands undergo forced degradation in different ranges and the maximum degradation has been observed in CTX4 , CTX1, CTX1 and CTX5 due to acid effect ,base effect ,oxidation effect and heat effect respectively.

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دراسة التفكك القسري لأصناف مختلفة من حقن السفتراكسون المتوفره في مدينة عدن

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الملخص

التفكك القسري هي عملية تنطوي على تفكك منتجات الأدوية والمواد الدوائية في ظروف أشد من الظروف العادية ومن ثَمٍّ يُولَد منتجات يمكن دراستها لتحديد استقرار جزيئات الأدوية.

جمعت خمسة أنواع من حقن المضاد السفتراكسون من السوق وتم دراستها تحت ظروف التفكك القسرية في الوسط الحمضي، الوسط القلوي، تأثير المواد المؤكسدة وتأثير الحرارة وقد استخدم جهاز السبكترومتر لمتابعة عملية التفكك.

أظهرت النتائج أنَّ كل أنواع المضاد الحيوي تتفكك بنسب متفاوتة تحت كل الظروف المدروسة خصوصا في الوسط القاعدي بنسب عالية.

الكلمات المفتاحية: التفكك القسري، حقن المضاد السفتراكسون، مدينة عدن.