Effect of buffers on the ertapenem stability in aqueous solution

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Key Word :Ertapenem, Buffer solution, Stability, PH

(Received :April 2014, Accept : Jun 2014)

Abstract

The stability of ertapenem, the active principle of Invanz®, was investigated in citrate (pH 3.50), acetate (pH 4.00 and 5.00), phosphate (pH 5.50, 6.50 and 7.50) and carbonate (pH 8.50) buffer solution at 30˚C and at an ionic strength of 0.5mol L^{-1}.

Changes in the concentration of ertapenem were determined by reverse-phase high performance liquid chromatography (HPLC) with UV-detection at 230 nm. The mobile phase used was phosphate buffer (20 mM) and acetonitrile (92.5:7.5) respectively.

The degradation of ertapenem obeyed pseudo first-order kinetics under all conditions employed in this study. The activation energy (Ea) for the degradation of ertapenem in hydrochloric acid (pH 1.20) was 62.7kJmol^{-1}. The catalytic effect of acetate and phosphate buffer species towards the degradation of ertapenem was also investigated. The buffer species have a significant catalytic effect on ertapenem degradation which increased with increase of buffer concentration. pH-rate profile of ertapenem displayed three regions; an acid catalytic reaction below pH 5.00, a pH-independent region between pH 5.00 to 7.50 and base catalysed above pH 7.50.

تأثير محاليل البفر على ثباتية الارتبنم في المحاليل المائية

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مفتاح الكلمات:ارتبنم ,محلول بفر ,الثباتية PH

الملخص

تم فحص ثباتيتة الارتبنم ,المادة الاساسية في دواء الانفانز, في محلول البفر الاستيتي (4 و 5) pH وفي محلول البفر الفوسفاتي (3.5 , 4.5 , 5.5) pH في درجة حرارة 30 ˚C .

التغيرات الحاصلة في تركيز الارتبنم كانت تفحص وتقاس من خلال جهاز HPLC بالموج الضوئي قدره 230 nm. محلول البفر الفوسفاتي كان الطور المتحرك بتركيز 20 mM مع مادة الاسيتونياتريل بنسبة (92.5:7.5)

التحطم في تركيز الارتبنم اتبع نظام الكاذب الاول في كافة التراكيز التي استخدمة في هذه الدراسة. الطاقة المفعلة لهذا التحطم في الارتبنم في حامض الهايدروليك كانت في (pH 1.20) 62.7kJmol^{-1} . لقد تم فحص

التاثير الاختزالي من قبل المواد المكونة للبفرالاستيتي و الفوسفاتي اتجاه مادة الارتبنم. لقد وجد أن هذه المواد المكونة لمادة البفر تأثير واضح وهذا التاثير كان يزداد مع زيادة تركيز البفر. ثلاث مناطق وجدت في الشكل الشخصي pH : الاولى منطقة الاختزال الحامضي اسفل pH 5 والثانية المنطقة المستقلة عن تأثير pH (5-7.5) والثالثة الواقعة تحت التأثير القاعدي وهي وهي فوق pH 7.5 .

1- Introduction

In aqueous solutions, carbapenems are unstable having two principle modes of degradation: hydrolysis and dimerization.(1, 2) Hydrolysis of the highly strained β-lactam ring system accounts for the instability of carbapenem antibiotics in water at high and low pH values and the degradation will be demonstrated by lactam ring opening(3). Dimerization happens only at high concentrations of carbapenem by undefined parallel intermolecular reactions (4). Sajonz et al. have demonstrated that the dimers are formed when an ertapenem sample concentration is high, i.e. ≥ 100 mg mL^{-1}. Carbapenems exhibit stability over a narrow pH range (5.5 – 7.5).

Ertapenem consists of a carbapenem ring with a 1β-methyl group and a unique anionic side chain. Influence of temperature, humidity on the stability of ertapenem has demonstrated in several studies.(5, 6) Sajonz et al. have found that an increase in the temperature of ertapenem solutions increased the overall degradation rate.(7)

Several studies have demonstrated the effect of pH on the degradation rates of different carbapenem drugs.(2, 7, 8) Obviously, increase or decrease in H^+ or OH^- concentration may affect the rate of degradation. In acidic solution they undergo β-lactam ring opening involving predominantly a pseudo first-order reaction. In alkaline solution they also undergo β-lactam ring opening and polymerization involving predominantly an overall second-order reaction. In neutral solution, they undergo nucleophilic attack by water molecules. Mendez et al. studied the catalytic effect of buffers on the degradation of imipenem.(9) The pH-rate profile of imipenem was interpreted kinetically at three important pH regions. One region was where a hydrogen-ion-catalyzed reaction took place (pH < 6.00). The second region was the independent pH region (pH 6.50-7.50), where the predominant reaction was the attack by water molecules, and third region where the reaction was hydroxide ion catalyzed (pH > 8.50).

A limited number of studies have described kinetic features of ertapenem in aqueous solution. Zajac et al. have found that ertapenem undergos a specific acid–base catalysis. The catalysis effect includes hydrolysis of ertapenem by hydrogen ion,

hydrolysis of ertapenem dianions by hydroxide ions and spontaneous hydrolysis of zwitter ions and dianions of ertapenem under the influence of the water.(10)

The present research deals with kinetic aspect of the degradation of ertapenem in buffer solutions of various pH's.

2- Experimental

2.1. Materials and reagents

Ertapenem for injection—INVANZ (Merck & Co. Inc., Whitehouse Station, NJ, USA) is a sterile, synthetic, white to off-white hygroscopic, weakly crystalline powder. Each vial contains 1.046 g of ertapenem sodium (equivalent to 1 g of ertapenem) and inactive ingredients: 175 mg of sodium bicarbonate and sodium hydroxide to adjust pH to 7.5. All other chemicals and solvents were obtained from Merck Pty, Australia and were of analytical or high-performance liquid chromatographic grade.

2.2. Methods

2.2.1. HPLC analysis

The high performance liquid chromatography instrument consisted of a high pressure pump (Waters 501, HPLC pump, Millipore USA), a Apollo C18 (5 micron) column of 150 mm length and ID of 4.6mm, a Rheodyne Model 7125 syringe loading sample injector with 20 µL sample loop, a ultraviolet detector (Waters 484, Tunable Absorbance Detector, Millipore, USA) and an integrator/printer (Hewlett Packard HP 3396A integrator). The mobile phase used was phosphate buffer (20 mM) and acetonitrile (92.5:7.5) at pH 6.5. The monitoring UV wavelength was 230 nm. The experiment was validated between analysis and day-day validation.

2.2.2. Stability study

Ertapenem product, Invanz®, was dissolved in buffer solutions separately to make a concentration of 1mg ml^{-1}. Buffer solutions (citrate at pH 3.50, acetate at pH 4.00 and 5.00, phosphate at pH 5.50, 6.50, and 7.50 and carbonate at pH 8.50) were employed to evaluate the degradation of ertapenem. These buffer solutions of varying concentrations were prepared to evaluate the rate of degradation of ertapenem at a constant pH, temperature (30ºC), and ionic strength of 0.5 $mol.L^{-1}$. Concentrations of residual drug were determined by HPLC. A plot of log percent drug concentration versus time was plotted to determine the rate of degradation and to find the rate constant of the reaction.

3- Results and discussion

The temperature dependence of ertapenem was studied in hydrochloric acid solution (pH 1.20) at 30, 22 and 13˚C. Degradation of ertapenem increased with increase of temperature. The Arrhenius plot was obtained as shown in Figure (1) and the apparent activation energy of ertapenem was calculated at pH 1.20 to be 62.7 $kJmol^{-1}$. Zajac et al. have found that the activation energy of ertapenem was between 50.18 and 64.83 kJ mol^{-1} for all reactions in acid and base.(11)

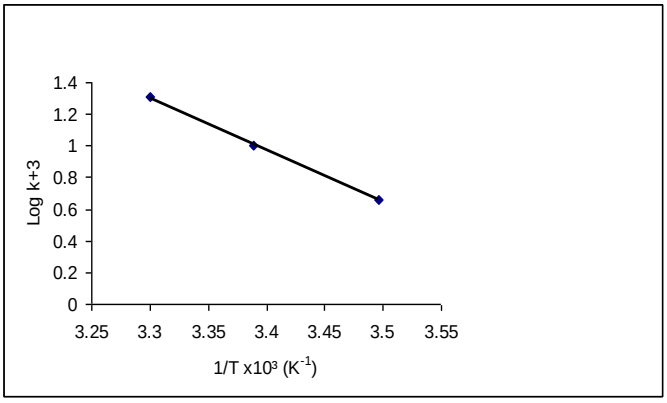

Figure (1) Thermal decomposition of 1mg mL^{-1} ertapenem sodium (Invanz®) in hydrochloric acid solution (pH 1.20), μ of 0.5 mol L^{-1} and at 13, 22 and 30˚C

A plot of log percent remaining concentration of ertapenem in different type of buffer solution versus time shows a linear relationship which indicates a pseudo first order rate of degradation of ertapenem.

The catalytic effect of the buffer systems used in the kinetic studies was determined by experiments at constant pH, temperature, ionic strength and drug concentration, the only variation being in the buffer concentration at a given pH. The catalytic effect of the buffer system was examined at 1mg mL^{-1} of ertapenem concentration, 30˚C, μ of 0.5 mol L^{-1} and at given pH values.

In order to calculate the catalytic rate constant values of each species of acetate and phosphate buffer solutions, a plot of the slopes of all reactions at pH values of 4.00, 5.00, 5.50, 6.50 and 7.50 versus the different buffer concentrations was plotted as shown in Figure (2) and (3). The slops of these plots in Figure (2) and (3) represent either the summation value of k_A [HAc] or k_B [Ac^-] at pH 4.00 and 5.00 Figur (2) or k_A [$H_2PO_4^-$] and k_B [HPO_4^{-2}] at pH 5.50, 6.50 and 7.50 Figure (3). The catalytic effect of acetate and phosphate buffers at different pH values increased linearly with the buffer concentration.

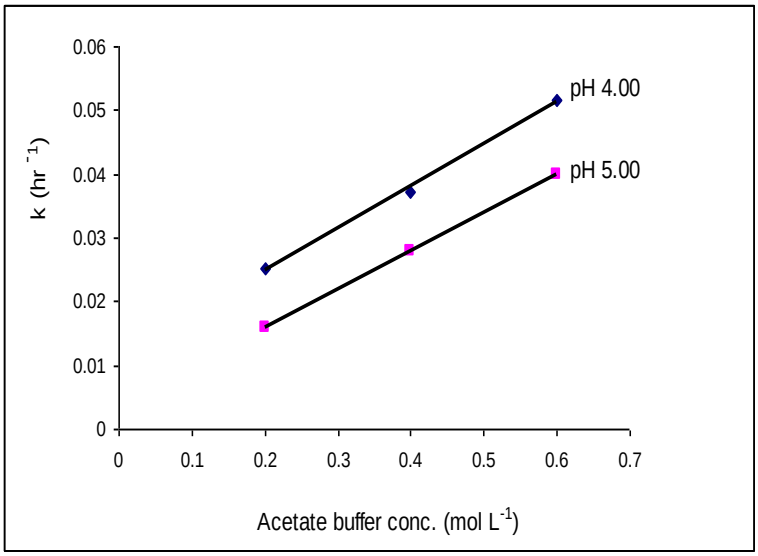

Figure (2) Catalytic effect of the acetate buffer solution on 1mg mL^{-1} ertapenem degradation at pH 4.00 and 5.00, µ of 0.5 mole L^{-1} and 30˚C.

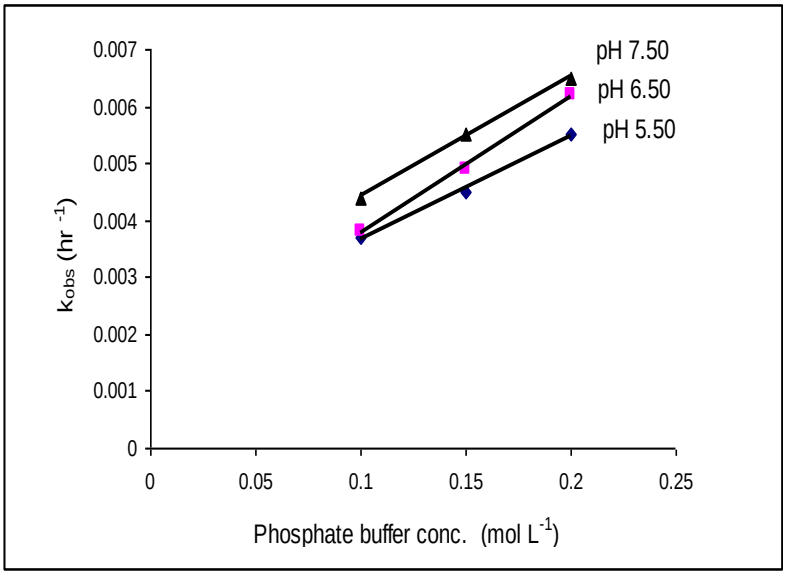

Figure (3) Catalytic effect of the phosphate buffer solution on 1mg mL^{-1} ertapenem degradation at pH 5.50, 6.50 and 7.50, µ of 0.5 mole L^{-1} and 30˚C.

The vales of k_A and k_B of acetate and phosphate buffer solutions were included in Table (1)

Table (1) Values of k_A and k_B of acetate and phosphate buffer solutions

Buffer Type	*k_A ($mol^{-1}.L.hr^{-1}$)*	*k_B ($mol^{-1}.L.hr^{-1}$)*
Acetaet	6.8×10^{-2}	5.6×10^{-2}
Phosphate	1.79×10^{-2}	2.19×10^{-2}

Hence the above data indicate that all species of acetic, sod.acetate, mono and di-hydrogen phosphate ions are catalytic towards the degradation of ertapenem. The most significant catalysis effect is being attributed to the acetic acid in acetate buffer solution and the mono-hydrogen phosphate ion in phosphate buffer solution.

At zero buffer concentration only specific acid-base catalysis (H^+ / OH^-) or a spontaneous water reaction occurs. The logarithms of the rate constants at zero buffer solutions ($k^{'}$) are plotted versus pH values in a range of 3.50 – 8.50. The pH-rate profile Figure (4) has a U-shape which is characteristic of reaction susceptible specific acid-base catalysis.

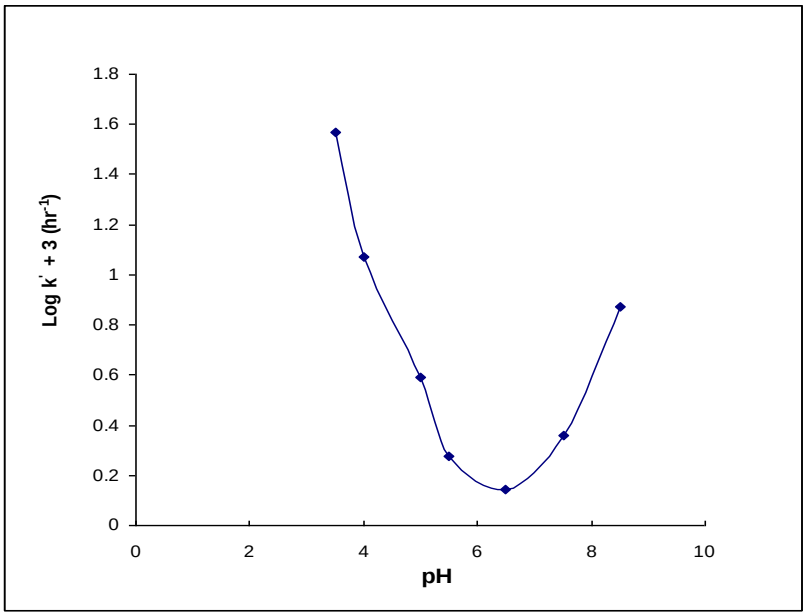

Figure (4) log $k^{'}$- pH profile of ertapenem degradation in aqueous solution at 30˚C and μ of 0.5 mol L^{-1}

The pH-rate profile of ertapenem can be demonstrated kinetically as follows: there were three important pH regions. One where the acid catalysed reaction took place, which was under pH 5.00, second one where the pH-independent region, which represents the pH range of 5.00 to 7.50 and the third one where the alkali catalysed reaction took place, which was above pH 7.50. The pH of maximum stability for

ertapenem in buffer free conditions is at pH range of 5.0 to 7.5. At pH range of 3.50 to 7.50, ertapenem (pKa_1 2.72, pKa_2 3.96 and pKa_3 7.06) will be influenced by the three pKa values therefore the catalytic rate constants of the water species in ertapenem hydrolysis are grouped under the constant k' according to the following Equation

$k' = k_{H2O} + k_H [H^+] + k_{OH} [OH^-]$

Where k_H and k_{OH} represent the second-order rate constants of proton and hydroxide ion-catalysed degradation respectively and k_{H2O} is the rate constant of water-catalysed degradation.

Conclusion

Degradation rate of ertapenem in buffer solution was shown to be significantly affected by general acid-base catalysis and increased linearly with increase the total concentration of the buffer solutions. Buffer species have a significant catalysis effect on degradation rate of ertapenem. Maximum ertapenam stability in aqueous solution was between pH 5.00 and 7.50,however, the best ertapenem stability at pH 6.50

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