Dual effect of changes in temperature and pH on stability of cefquinome *in vitro*

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Abstract

The current study includes the dual effect of changes in temperature and pH on stability of cefquinome *in vitro*. Cefquinome was exposed to different phosphate buffer solution with a pH of 6,7 or 8 and each one was exposed to different temperatures which were 30̊C,50̊C or 70̊C in a water bath during 24 hours. Samples were collected after dissolving, after exposure to different pH and after 1,3,6,12 and 24 hours of exposure to different temperatures and pH values. Microbiological assay was used to analyze the samples. The results showed that there was a significant decrease in cefquinome concentrations(antibacterial activity) in alkaline medium with increasing temperature within the time. In conclusion cefquinome is affected by increasing temperature in alkali medium which causes a decrease in its concentration that will affect efficacy of cefquinome due to the degradation process.

Keywords: Degradation, Biological assay, Cephalosporins
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التأثير المزدوج لتغير درجات الحرارة والأس الهيدروجيني (ألباها) على ثباتية عقار السفكوينوم في الزجاج

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الخلاصة

أجريت الدراسة الحالية لمعرفة التأثير المزدوج لتغير درجات الحرارة والأس الهيدروجيني (ألباها) على ثباتية عقار السفكوينوم في الزجاج، إذ تم تعريض عقار السفكوينوم إلى محلول دارئ الفوسفات بدرجات باها مختلفة ٦ أو ٧ أو ٨ وكل من هذه المحاليل تم تعريضها إلى درجات حرارة مختلفة أيضا ٣٠ أو ٥٠ أو ٧٠ مئوي في حمام مائي لمدة ٢٤ ساعة. تم جمع العينات بعد الإذابة مباشرة وبعد التعرض لمختلف ألباها وبعد ١ و ٣ و ٦ و ١٢ و ٢٤ ساعة من التعرض لمختلف درجات الحرارة وألباها. وقد استعملت طريقة التحليل المايكروبايولوجية لتحليل العينات. أظهرت النتائج وجود انخفاض معنوي في تركيز عقار السفكوينوم (الفعالية المضادة للبكتريا) في الوسط القاعدي بزيادة درجات الحرارة ومع مرور الزمن. يستنتج من ذلك بأن عقار السفكوينوم يتأثر بزيادة درجات الحرارة في الوسط القاعدي والتي أدت إلى انخفاض التركيز وعليه قلة فعالية عقار السفكوينوم نتيجة عملية التحلل الكيميائي.

Introduction

Cefquinome, an aminothiazolyl cephalosporin is the first member of fourth-generation cephalosporins which have been developed for use in veterinary medicine (1,2). The *in vitro* and *in vivo* efficacy of this drug against a wide range of Gram-negative and Gram-positive bacteria has been demonstrated (1). Cefquinome is effective against causative agents of respiratory tract infections, diarrhea and mastitis in cattle (3-7). Pharmaceutical dosage forms should be stable during preparation, administration and action. β-Lactam antibiotics (penam analogues, cephalosporins and carbapenems) are susceptible to degradation both in aqueous solutions and in the solid state (8). Determination

of cephem analogs is the result of different physical and chemical factors activities.

The need to develop a stability-indicating method using stress degradation has been recommended by International Conference of Harmonization(ICH) (9). In practice, the effects of pH and temperature changes on drug stability are often used in such studies. The results of such studies are of great importance in the estimation of drug shelf life and the effect of degradation products on decreasing efficacy and possibly causing toxicity. They may also serve as guides for better drug design, drug formulation and drug analysis (10).

During stress tests, the effect of temperature and air humidity should be determined in solid state. For solutions, the effect of temperature, light, oxidizing agent, buffer pH and infusion liquid need to be analyzed. The impact of biochemical processes on the formation of metabolites has to be considered as well. Finally, the chemical structure and toxicity of principal degradation products, impurities and metabolite(s) should be established (8). Therefore, the aim of the present work was to investigate the effects different pH and temperatures on stability of cefquinome within time of exposure.

Materials and methods

Chemicals

Cefquinome sulfate (Cobactan4.5%) was obtained from Intervet Nederland B.V. Company for injection and its diluent benzyl alcohol (E1519) 10%. Phosphate buffer solution at different pH values 6, 7 or 8.

Experimental protocol

Cefquinome was prepared by weighing 4.5 mg and completing the volume to10 ml of the special diluent for the product (10% benzyl alcohol). Different phosphate buffer (PB) solutions at pH 6, 7 or 8 had a final concentration of cefquinome 9 µg/ml were exposed to a different temperatures 30, 50 or 70 °C during 24 hours in a water bath. Samples of each, were collected as follow : after dissolving (without PB),direct exposure (to PB), 1, 3, 6, 12 and 24 hours of exposure to different PB solutions and temperatures.

Microbiological assay

Cefquinome concentrations in samples were determined by microbiological assay method (11) using *Micrococcus luteus* (American Type Culture Collection ATCC 272) as an indicator organism (12). Standard curve of drug concentrations were 1.125, 2.25, 4.5, 9 and 18 µg /ml. Six wells were made at equal distances in standard petri-dishes containing 15 ml seeded agar. The plates were incubated at 37°C for 24 h.

The inhibition zone diameters were measured and the cefquinome concentrations in the test samples were extrapolated from the standard curve. Semi-logarithmic plots of the inhibition zone diameter versus standard cefquinome concentrations were linear with typical correlation coefficient of 0.990 (for the standard curve).

Statistical analysis

Samples were presented as mean± standard error (SE). Statistically significant differences of samples estimated on the basis of concentrations measured by analysis of variance (ANOVA) using least significant difference (LSD) by the aid of the computer program SPSS.

Results

Effect of pH on stability of cefquinome at different temperatures showed the decrease in concentrations of the drug by increasing pH and temperature within the time, and the lesser effect of degradation at pH 6 with temperature 30**°C**, while the largest one was at pH 8 with temperature 70**°C** (Table 1).

The concentrations of cefquinome was absent at pH 6 and 70**°C** after 12 hours of exposure, whereas at pH 7 and 70**°C** the drug was absent after 6 hours of exposure and the largest effect was at pH 8 where the concentration of drug disappeared after 12 hours at 50**°C** temperature and after one hour at 70**°C**.

The degradation ratio (DR) (%) of cefquinome at pH 6 was 24% at 30**°C**, 52% at 50**°C** and 100% at 70% after 24 hours of exposure, while at pH 7 the DR was 36% at 30**°C**, 60% at 50**°C** after 24 hours of exposure, while the DR was 100% at 70**°C** after 6 hours of exposure (Table 2). At pH 8 the DR was 50% at 30**°C** after 24 hours of exposure, while the DR was 100% at 50**°C** after 12 hours and at 70**°C** after one hour of exposure (Table 2).

Discussion

The stability of a pharmaceutical product is defined as the capability of the product to retain its efficacy, properties and characteristics throughout its shelf life (13). One of the most important types of stability is chemical stability which includes hydrolysis. Amides are generally more stable to hydrolysis than esters. In general, the rate of hydroxyl ion-catalyzed reaction of amides is greater than the rate of proton-catalyzed hydrolysis (14). Cephalosporins are amides in which the amide bond is part of strained four-membered β-lactam ring. The decomposition of these compounds is catalyzed by solvent, hydroxide ion, and many buffer species and thus is unstable to be formulated as solutions (13,15).

Cephalosporins are well known to degrade in alkaline media to give penicilloic acid (16). In this study, degradation rate was found to be [OH^-] dependant, suggesting that the OH^- is playing the role of nucleophile

(intramolecular hydrolysis) to give the proposed degradation product (10).

The present study agrees with previous studies, that the degradation rate of cefquinome increase during acidic hydrolysis, basic hydrolysis and exposure to increasing temperature (8), but does not agree with some other results (10), as cefquinome was found to be stabile in acidic pH (2-6) even at temperature above 80°C. In present study cefquinome was not stable at pH 6 within the time when increasing temperature. This may be due to the way of analysis, because in the present study it was carried out by using a microbiological assay method to determine the active concentration (antimicrobial activity), whereas the previous study used HPLC method to analyze the samples.

Table 1: Concentrations of cefquinome (µg /ml) at different pH values and temperatures with progressing time

Time (hour)	pH								
	6			7			8		
After dissolving	8.60±0.20 Aa			8.70±0.15 Aa			8.73±0.17 Aa		
After exposure	8.46±0.23 Aa			8.23±0.37 Aa			8.26±0.06 Aa		
After 1 hour	8.03±0.32 Aa	7.40±0.06 Ab	6.87±0.32 Ab	7.10±0.32 Ab	6.33±0.17 Ab	4.47±0.42 Bb	6.87±0.37 Ab	4.97±0.13 Bb	0.00±0.00 Cb
After 3 hours	7.93±0.35 Aa	7.03±0.03 Bb	5.27±0.87 Bc	6.40±0.10 Bc	5.77±0.15 Bb	3.00±0.42 Dc	6.77±0.64 Bb	4.17±0.07 Cc	0.00±0.00 Eb
After 6 hours	7.90±0.32 Aa	6.00±0.30 Bc	3.17±0.17 Dd	5.97±0.03 Bc	5.00±0.06 Cc	0.00±0.00 Ed	6.20±0.42 Bb	2.60±0.23 Dd	0.00±0.00 Eb
After 12 hours	7.36±0.41 Aa	5.23±0.13 Bd	0.00±0.00 De	5.73±0.07 Bc	4.30±0.15 Cd	0.00±0.00 Dd	5.23±0.64 Bc	0.00±0.00 De	0.00±0.00 Db
After 24 hours	6.53±0.29 Ab	4.13±0.33 Ce	0.00±0.00 De	5.57±0.03 Bc	3.47±0.17 Ce	0.00±0.00 Dd	4.37±0.43 Cd	0.00±0.00 De	0.00±0.00 Db
Temp.	30°C	50°C	70°C	30°C	50°C	70°C	30°C	50°C	70°C

Values are mean ± SE, n=3, P<0.05. Capital letters indicate the differences between treated groups (horizontally). Small letters indicate the differences within treated groups (vertically). Zero values indicate the complete disappearance of drug.

Table 2: The degradation ratio (%) of cefquinome

Temperature	pH		
	6	7	8
30°C	24% after 24 hours	36%after 24 hours	50% after 24 hours
50°C	52% after 24 hours	60% after 24 hours	100% after 12 hours
70°C	100% after 24 hours	100% after 6 hours	100% after one hour

Conclusions

The results of the current study showed that cefquinome solution degraded via hydrolysis process that appears to be [OH^-] and temperature dependent. The results indicated that the degradation rate and subsequently the $t_{1/2,}$ decrease with increasing OH^- concentration and elevate temperature. Cefquinome is stable in acidic medium at pH 6 and low temperature, but as these two factors increase the drug start to degrade, These findings suggest that the formulation of this drug in liquid form should be at pHs on the acidic side (less than 6).

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