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Research article

Stability studies on Lincomycin HCl using validated developed second derivative spectrophotometric method

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Key words: Lincomycin; Stability; Second derivative; Stress conditions; Hydrolysis.

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Abstract

Objectives: A previously developed second derivative spectrophotometric method was applied for the stability studies of lincomycin under stress conditions in accordance to VICH guidelines. **Method:** The studied factors were pH, alkali, acid, temperature and light. **Results:** A constructed pH profile revealed the degradation rate dependence on [OH-]. Arrhenius plot obtained at pH 10 was linear between 70°C and 100°C. The estimated activation energy of the hydrolysis was found to be 17.4 kcal mol^{-1}. Photochemical studies were also conducted. **Conclusion:** Lincomycin was found unstable in alkali and high temperature.

Introduction

Veterinary drugs are very important for our health as they have indirect effect through food (poultry& Livestock). One of the most important veterinary drugs is antibiotic which has been used for years to treat and control animals' disease. The other major use for farm animals is the employment of antibiotics for animal growth promotion which is highly controversial in terms of its safety not only for the animals but also for the people who consume these animals' products. The question of increased antibiotic resistant in humans, due to antibiotic use in animals, is contested [1].

Due to the high increase in resistance for antibiotics, it is absolutely necessary to do a severe quality control of veterinary commercial in terms of both qualitative and quantitative analysis [2].

Lincomycin (Figure 1) is a lincosamide antibiotic that comes from the actinomycete *Streptomyces lincolnensis*. It is a narrow spectrum antibiotic with activity against Gram-positive and cell wall-less bacteria including pathogens of *Streptococcus*, *Staphylococcus,* and *Mycoplasma* [3]. Lincomycin is used to treat severe bacterial infections in patients who cannot use penicillin antibiotics [4]. Literature review revealed many chromatographic and spectrophotometric methods for the determination of lincomycin in pharmaceutical formulations and biological fluids [5-10].

Derivative spectrophotometry is an analytical technique, which differentiates the normal spectrum by mathematical transformation of spectral curve into a derivative (first- or higher derivatives). Derivative spectrophotometry has been widely applied in the analysis of different pharmaceutical dosage forms. It solves the problem of analysis associated with drug combination, stability studies of drug and degradation products, drug impurities, and interference of excipients in drugs [11].

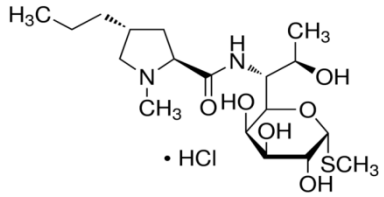

Figure 1. Lincomycin HCl structure

Although one of the reported methods was applied for the stability studies of lincomycin, it described different and drastic conditions [9].

We have been involved in development of spectrophotometrical methods for the analysis of drugs [11]. Recently we have developed a new method for the routine analysis of lincomycin [12], and herein we apply it for the stability studies on lincomycin in bulk and pharmaceutical forms.

Experimental

Materials

Standard and sample

Lincomycin standard was kindly provided by SFDA (Saudi food and drug administration), KSA. Lincocin® injection solution, 600mg/2ml, Pfizer pharmaceutical USA.

Reagents
Sodium hydroxide: BDH, Poole, England. Hydrochloric acid: BDH, Poole, England. Disodium orthophosphate: BDH, Limited Poole, England. Citric acid monohydrate: Lobachemie, India Mcllvaine universal buffer (pH range 2.2- 8) and phosphate buffer (pH range 9-11) were prepared according to the reported methods [13, 14].

Instruments and apparatus
All UV measurements were done on UV-1800. Model UV-1800ENG240V, Shimadzu Corporation, Koyoto, Japan. Heating was performed using Water bath Temperature regulated**:** Model: YCM-010E, Germany. Weighing was done using Balance: Kern ALS 120-4, Germany.

Preparation of stock solution
Distilled water was the diluent solvent used in all the experimental work.

Lincomycin standard solution
An accurately weighed quantity of lincomycin standard (300 mg) was dissolved in 30ml distilled water. The solution was transferred into 100 ml volumetric flask and volume was then completed to mark with the diluent solvent. 1 ml of the resultant solution was further diluted to 100 ml (solution A; 30 µg/ml)

Lincomycin sample solution
One ml of lincomycin injection solution was accurately pipetted and transferred into100 ml volumetric flask. The volume was completed to mark with the solvent. 1 ml was further diluted to 100 ml with the solvent (solution B; 30 µg/ml).

Procedures
Photodegradation of lincomycin in glass bottles
Five ml of solution B was placed in stoppered glass tube, diluted to 10ml with the solvent. The solution was then subjected to irradiation with the sunlight during mid-day for time ranging between 1-6hours. Degradation was then monitored by the developed second derivative spectrophotometric method.

Effect of alkali and acid on stability of lincomycin
Three ml of freshly prepared solution B was transferred into a set of stoppered glass tubes (five tubes). 1ml of 1M sodium hydroxide solution was added to each tube. The volume of one glass tube was completed to 10ml with the solvent and the second derivative spectrum was then recorded. The four stoppered glass tubes were heated in a boiling water bath at 10 minutes heating time interval. The volumes were then completed to 10ml with solvent and the second derivative spectra were recorded.

The above procedure was repeated using 0.5M NaOH, 2M NaOH and 1M HCl solution instead of 1M NaOH to study the effect of different alkali concentration and acid on lincomycin stability.

Effect of pH on the stability of lincomycin
Three ml of freshly prepared solution B was transferred into three sets of stoppered glass tubes (11 tubes each).One ml of the phosphate buffer (pH values 2.2, 3, 4, 5, 6, 7, 7.4, 8, 9, 10 and 11) was added to each tube. The solutions were heated in boiling water bath at 10 minutes heating time interval. The reaction was then quenched by cooling, and the volumes were completed to 10 ml with the solvent. Degradation of lincomycin was monitored by the developed second derivative spectrophotometric method. The rate constant for each pH was calculated from the plot of log [% remaining drug] *vs* time.

Effect of temperature on the stability of lincomycin
Three ml of freshly prepared solution B was transferred into three sets of stoppered glass tubes (four tubes each). One ml of phosphate buffer (pH 10) was added to each tube. The solutions were heated at 70ºC, 80ºC and 100ºC within suitable time intervals (10 minutes). The reaction was then quenched by cooling and the volumes were completed to 10 ml with the solvent.

Results and Discussion

Many drugs contain functional groups increasing their susceptibility to chemical degradation such as hydrolysis. This will lead to loss of potency, change in physical appearance and/or increase of toxicity [15].
Different organizations; ICH & VICH, described the requirements and guidelines for the assessment of drug quality and stability studies. The results of which are of great importance in the estimation of drug shelf life and the effect of degradation products. Also gives guides for better drug design and formulation.
Stress testing also helps in suggesting a reliable method of analysis of a drug simultaneously with its possible degradation products.
Although the reported first and second derivative methods were applied for the quantitative analysis of lincomycin, the second derivative method was selected to study the degradation behavior of lincomycin as the first derivative method failed to give reproducible results for the stability study.

Photo-degradation
The effect of light is often considered an important factor in drug stability. Great stability problems arise by light of the shorter wave length [16].The photodegradation study on tested drugs was intended to obtain useful information about the sensitivity of the drug to light. Conditions of irradiation were controlled to study the effect of light and

not energy (heat). The decomposition of irradiated drug solutions with sunlight for about 6 hours was monitored using second derivative spectrophotometry. The solutions remained stable and there was no change neither in spectra nor in the concentrations. This reflected the stability of drug solutions under these conditions during the time intervals studied. Otherwise, the light effect to be observed might need high drug concentration or longer exposure periods.

Effect of alkali and acid

The developed second derivative spectrum of lincomycin showed absorption peak at 230 nm [12]. Treatment of this solution with alkali resulted in spectral changes.

Therefore, the effect of different alkali concentrations coupled with different heating time intervals was studied. The study condition which gave measurable degradation rate with good linearity was found to be 1M NaOH with 10 minutes heating time interval. The UV scanning of the second derivative spectra of alkali-treated lincomycin solutions reflected a decrease in its peak at 230 nm (Figure 2).

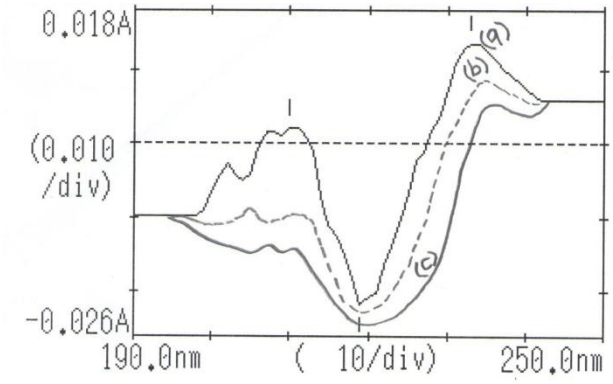

Figure 2. Second derivative spectrum of Lincomycin with 1M NaOH in the following time intervals: a: 20 minutes, b: 30 minutes, c: 40 minutes.

The degradation rate constant and subsequently the $t_{½}$ and t_{90} were calculated from the linear regression data of log % remained drug vs heating time for three time intervals (Figure 3, Table 1). The degradation rate was found to increase with increased heating time. Using hydrochloric acid, the concentration and spectrum of lincomycin were not affected with the different concentrations of acid even at high temperatures.

Table 1. Slope, K_{obs}, $t_{½}$ and t_{90} for lincomycin degradation in 1M NaOH at 100°C

Slope	K_{obs}	$t_{½}$ (minutes)	t_{90} (minutes)
0.0059	0.0136	51.00	7.70

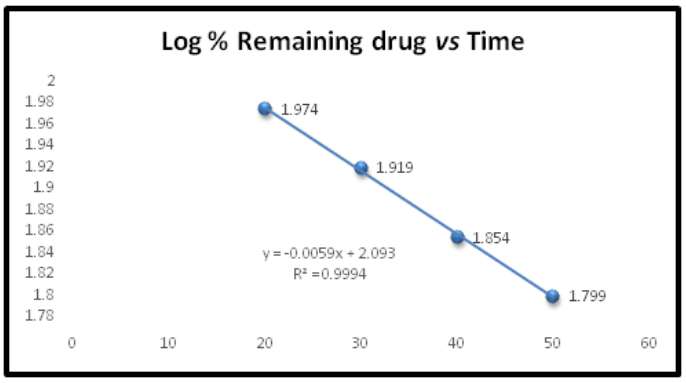

Figure 3. Effect of sodium hydroxide (1M) in the degradation of Lincomycin.

Effect of pH

Different pH values were tested to study their effect on lincomycin stability. A plot of log K_{obs} (degradation rate constant) versus pH values gave a positive slope on the alkaline side. This suggested first order dependence of the degradation rate on [OH−]. The obtained pH profile (Figures 4) resembles subtype BCD in the generalized pH polygon, where K_{obs} increases, and hence $t_{1/2}$ decreases, at high pH values [17].

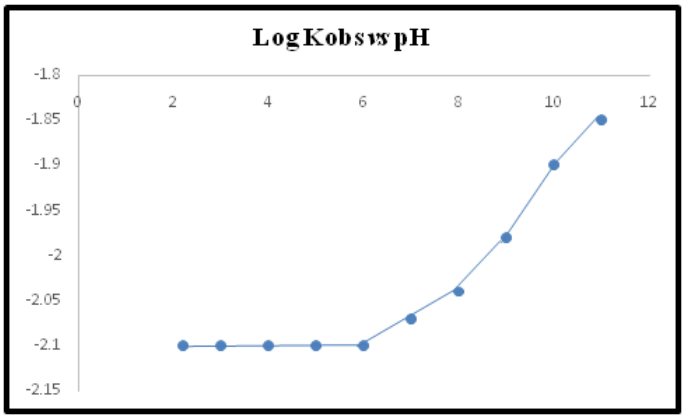

Figure 4. Effect of pH on the degradation rate of lincomycin at 100°C.

Effect of temperature

The developed second derivative method was applied to monitor the time course decomposition in phosphate buffer (pH 10) at temperatures of 70˚C, 80˚C, and 100˚C. The linearity of the constructed plots at different temperatures (log % remaining drug versus time) reflected the dependence of the decomposition reaction on temperature (Figure 5). The activation energy (Ea) was calculated (17.4 Kcal mol−) using Arrhenius plot (Figure 6), which was then utilized to calculate the shelf life t_{90} and half life $t_{1/2}$ for lincomycin at different temperatures (Table 2).

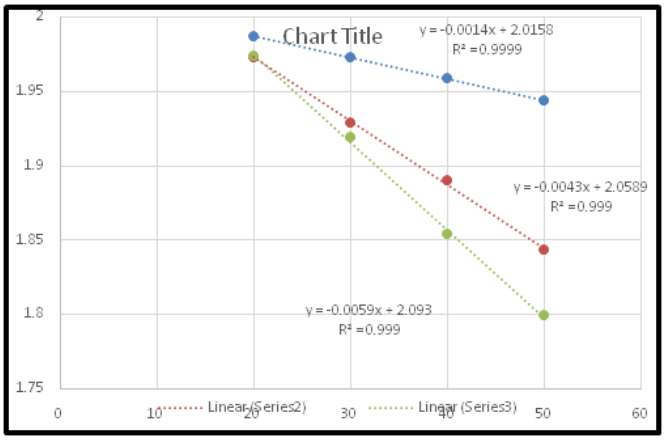

Figure 5. Time-course of the decomposition of Lincomycin in phosphate buffer pH10 at different temperatures.

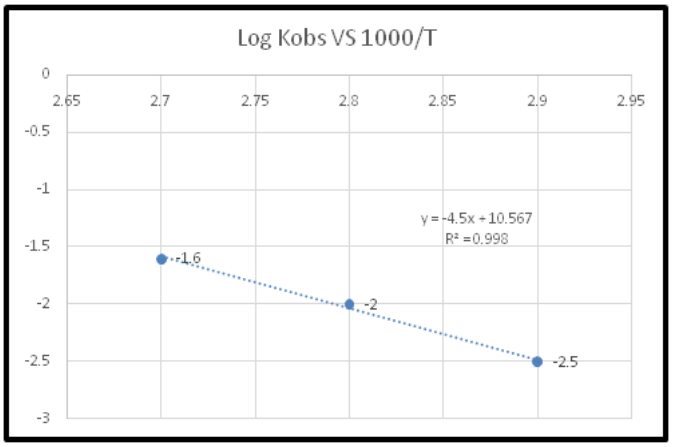

Figure 6. Arrhenius plot for degradation of Lincomycin in phosphate buffer pH 10 and temperatures 70°, 80° and 100°C.

Table 2. Kobs , $t_{1/2}$ and t_{90} values for Lincomycin degradation at pH 10 at different temperatures

Temp.	K_{obs} (min^{-1})	$t_{1/2}$ (0.693/K)	t_{90} (0.105/K)
70ºC	3.2×10^{-3}	216.60	32.80
80ºC	9.9×10^{-3}	70.00	10.60
100°C	0.0136	51.00	7.70

Conclusion

The developed second derivative spectrophotometric method was applied for the stability studies on lincomycin where the potential degradation was investigated in accordance with VICH guidelines. It was found unstable in alkali, high temperature and pH > 7.3. On the other hand, acid, light and otherwise pH did not cause its degradation.

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