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Original Research Paper

Degradation kinetic study of lysine in lysine hydrochloride solutions for injection by determining its main degradation product

*Mengying Tao, Meng Zhu, Chunnuan Wu, Zhonggui He**

Shenyang Pharmaceutical University, No. 103, Wenhua Road, Shenyang 110016, China

ARTICLE INFO

Article history:
Received 8 April 2014
Received in revised form
17 July 2014
Accepted 21 August 2014
Available online 28 August 2014

Keywords:
Lysine hydrochloride
Lysine lactam
Degradation kinetics
Shelf life

ABSTRACT

A limited number of researches have been reported to apply the Arrhenius equation to study the relationship between drugs and its degradation products so far. In the present work, the thermal degradation kinetics of lysine hydrochloride solutions for injection, the special solvent for ademetionine 1, 4-butanedisulfonate (SAM) for injection, was investigated at selected temperatures and pH values. The main degradation product of lysine was separated, purified, and confirmed as lysine lactam. A reversed-phase high performance liquid chromatographic (RP-HPLC) method without derivation was developed for the simultaneous determination of lysine and lysine lactam. The results confirmed that both the lysine degradation and lysine lactam generation followed zero-order reaction kinetics. The degradation and generation rate constants increased with increasing temperatures and decreasing pH values. The temperature-dependent degradation and generation reaction could be sufficiently modeled on the Arrhenius equation with the activation energy of 80.14 and 83.22 kJ/mol, respectively. Meanwhile, a linear relationship existed between the amount of lysine degradation and lysine lactam generation since the approximate activation energy. Considering there could be other side effects, we established an upper limit of lysine lactam (500 μg/ml), as the acceptable criteria for stability to estimate the shelf life together with lysine, which made the prediction more accurate and credible. Extrapolation data demonstrated that the lysine hydrochloride solutions for injection could be stable for two years stored at room temperature.

1. Introduction

Lysine, one of the essential amino acids, plays an important part in promoting human development, enhancing immune function and improving the function of the central nervous tissue [1]. Meanwhile, lysine also has a positive effect on the absorption of calcium and the formation of collagen with other nutrients [2]. Additionally, lysine is generally used as a

* *Corresponding author*. Tel./fax: +86 24 23986321.
E-mail address: hezhgui_student@aliyun.com (Z. He).
Peer review under responsibility of Shenyang Pharmaceutical University.
http://dx.doi.org/10.1016/j.ajps.2014.08.012

nutritional supplement to make up the possible deficiencies of lysine in humans [3]. Although lysine shows great promise, it is extremely unstable under normal conditions. Hence, the natural basic amino acid L-lysine generally exists in the form of crystalline salts of acid and basic bases, especially in the hydrochloride and acetate forms, to improve the stability.

The determination of lysine is becoming increasingly important in clinical analysis, biochemistry and pharmaceutics. Several methods are reported for the lysine determination based on spectrophotometry, potentiometric titration, thin layer chromatography, liquid chromatography, micellar liquid chromatography and hydrophilic interaction chromatography [4–9]. The determination of lysine by flow injection analysis techniques [10] has also been described. Even though there are so many methods available, there are still problems need to be solved, including the complex sample pretreatment process, time-consuming analysis and the uncommon instrumentations. As a zwitterionic compound, the reversed-phase chromatography of lysine with buffered mobile phase at different pH values usually presents typically poor retention [11]. However, mobile phase containing ion-pairing reagents [12] can improve retention of such situations, which makes the rapid and direct determination of underivatized lysine possible.

Since the poor stability of Ademetionine 1, 4-butanedisulfonate (SAM) under weakly acidic and alkaline conditions, it is infeasible to directly adjust the pH of the solution to neutral during the preparation of SAM for injection. Hence, a special solvent, lysine hydrochloride solutions for injection need to be prepared as pH modifiers to adjust the pH of the SAM solution before injection to improve the stability during processing and storage. However, our research found that a significant degradation product had generated in solutions for injection after sterilization, and no information was available on the structure and possible generation pathways of the degradation product. To guarantee the quality of solutions for injection, the contents of both lysine and the degradation product should be used as the control parameters. Also, since few studies have investigated the kinetic stability of lysine and the degradation product to date, a thermal degradation kinetic study on the lysine hydrochloride solutions for injection should be conducted applying the Arrhenius equation to quantify the effect of temperature on the product quality and to predict the shelf life [13].

In this study, we attempted to separate and confirm the main degradation product in lysine hydrochloride solutions for injection, and to develop a sensitive RP-HPLC method to rapidly detect lysine and the degradation product. Our efforts focused on the kinetic investigation of the lysine degradation and the main degradation product generation in solutions for injection under various conditions. The aim of the present work was to study the thermal stability of lysine hydrochloride solutions for injection, and determine the kinetic parameters of lysine degradation and the degradation product generation, with respect to the effects of pH value and temperature; and to apply the Arrhenius equation for predicting the shelf life of solutions for injection during storage and/or thermal processing.

2. Materials and methods

2.1. Materials

Lysine hydrochloride was purchased from Kyowa Amino Acid Co., Ltd. (Shanghai, China; Purity, 100.1%). Lysine lactam standard substance was purchased from Sigma–Aldrich (Shanghai, China; Purity $\geq$ 97.0%). Methanol of HPLC grade was purchased from Concord Technology Co., Ltd (Tianjin, China). All other materials were analytical grade quality. All solutions were prepared using distilled water throughout the study.

2.2. Preparation of lysine hydrochloride solutions for injection

To study the thermal stability, lysine hydrochloride solutions for injection with a lysine concentration of 83.5 mg/ml were prepared by the following methods. The accurately weighed lysine was dissolved in 60% of the total volume of water for injection, and 0.1% (w/v) activated carbon was added with stirring. After stirring at room temperature for 15 min, the solution was filtered through 0.45 μm membrane to remove the activated carbon. Next, the pH of the solution was adjusted with sodium hydroxide solution to pH 10.3, and then water for injection was added to volume. The obtained solution was filtered again using a 0.22 μm membrane filter to make the solution clear and sterile. Finally, the solution was nitrogen-filled sealed and autoclaved at 121 °C for 12 min.

2.3. Separation, purification and characterization of the main degradation product

Since the rare information available on the degradation product in lysine hydrochloride solutions for injection, method for separating and purifying the degradation product was designed as follows: the solvent was removed from the lysine hydrochloride solutions for injection by vacuum evaporation to form a concentrated mixture of lysine and the main degradation product. Subsequently, the mixture was dissolved in methanol and lysine was filtered out because of its poor solubility. The filtrate was collected and dried in a vacuum rotary evaporator. The resulting crude product was loaded onto a silica gel column, with n-propanol-ammonia (5:1) as eluent. The fractions were collected and sprayed to yield the degradation product-rich powders.

The structure of the main degradation product was characterized by proton nuclear magnetic resonance (^{1}H NMR) spectra and infrared spectra, applying a Bruker AV-600 MHz NMR spectrometer and a Bruker IFS-55 infrared spectrometer, respectively.

2.4. Degradation kinetic modeling of lysine hydrochloride solutions for injection

The thermal stability of the lysine hydrochloride solutions for injection was studied at selected temperatures (60, 80, 90 and 100 °C). The pH 10.3 lysine hydrochloride solutions for injection were placed in a thermostatic water bath (Zhengzhou Greatwall Scientific Industrial and Trade Co. Ltd., China)