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Short communication

Development and validation of indirect RP-HPLC method for enantiomeric purity determination of D-cycloserine drug substance

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ABSTRACT

A new chiral purity method was developed for D-cycloserine (D-cys) by reverse phase HPLC and validated. Chiral derivatizing reagents, viz., o-phthalaldehyde and N-acetyl-L-cysteine were utilized in this method. The resultant diastereomers were resolved using Zorbax SB Phenyl HPLC column under isocratic elution. A mobile phase of 95:05 (v/v), 20 mM Na_2HPO_4 (pH 7), and acetonitrile, respectively, was used with the flow rate of 1.0 mL/min and UV detection at 335 nm. The method development with different chiral stationary phases and chiral derivatization reagents were also investigated. The stability of diastereomer derivative and influence of organic modifier and pH of the mobile phase were studied and optimized. The stability-indicating capability of the method was established by performing stress studies under acidic, basic, oxidation, light, humidity and thermal conditions. The detection and quantitation limit of L-cycloserine (L-cys) were 0.015 and 0.05% (w/w), respectively. A linear range from 0.05 to 0.30% (w/w) was obtained with the coefficient of determination (r^2) 0.998. The recovery obtained for L-cys was between 92.9 and 100.2%. This method was applied successfully in pharmaceutical analysis to determine the content of L-cys in D-cys bulk drug.

1. Introduction

D-Cycloserine or D-4-amino-3-isoxazolidinone [1] is a broad-spectrum antibiotic which is used in the treatment of tuberculosis (Fig. 1a). The most important property of D-cycloserine (D-cys) is the inhibition of the growth of *Mycobacterium tuberculosis*. The worldwide resurgence of tuberculosis, the emergence of multiply drug-resistant tuberculosis and the problematic use of available drugs required to treat these infections, have resulted in the application of D-cys as a second line drug for the treatment of tuberculosis [2]. Official monographs available for D-cys in United States Pharmacopoeia and The International Pharmacopoeia refers only assay or impurities determination by LC but chiral purity estimation was not reported [3,4]. Also, there is no method published so far for the enantiomeric purity of D-cys by HPLC except a capillary electrophoresis–mass spectrometry (CE–MS) method utilizing crown ether as a chiral selector [5]. According to International Conference on Hormonization (ICH), Food and Drug Administration (FDA) and European Medicines Agency (EMEA) guidelines, any identified impurity must be controlled to less than 0.15% with respect to (D-cys) drug substance [6–8]. Hence, the present investigation was initiated with the objective to develop a simple, sensitive and stability-indicating method for L-cys enantiomer impurity determination in D-cys drug substance by reverse phase (RP) HPLC.

Amylose, cellulose, crown ether and protein based chiral stationary phases were investigated for the direct estimation of L-cys in D-cys by HPLC but no good separation could be achieved. Hence, indirect [9,10] chiral separation of enantiomers after derivatization with chiral Marfey's reagent, Sanger's reagent and o-phthalaldehyde (OPA) with N-acetyl-L-cysteine (NAC) were evaluated [11–15]. Derivatization with OPA and NAC has been utilized in this study for the determination of L-cys in D-cys drug substance. OPA–NAC has the advantage of rapid and complete derivatization at room temperature [16,17] itself. Selection of reverse phase HPLC column, mobile phase composition and pH of the mobile phase were found to play a vital role in the separation and sensitivity of the developed method. The proposed RP-HPLC method with UV detection has been validated using ICH and USP [18,19] guidelines as references.

2. Experimental

2.1. Chemicals and reagents

HPLC grade acetonitrile, AR grade Na_2HPO_4 and *ortho*-phosphoric acid (88%) were purchased from Merck (Mumbai, India). The water used was from a Milli-Q purification system,

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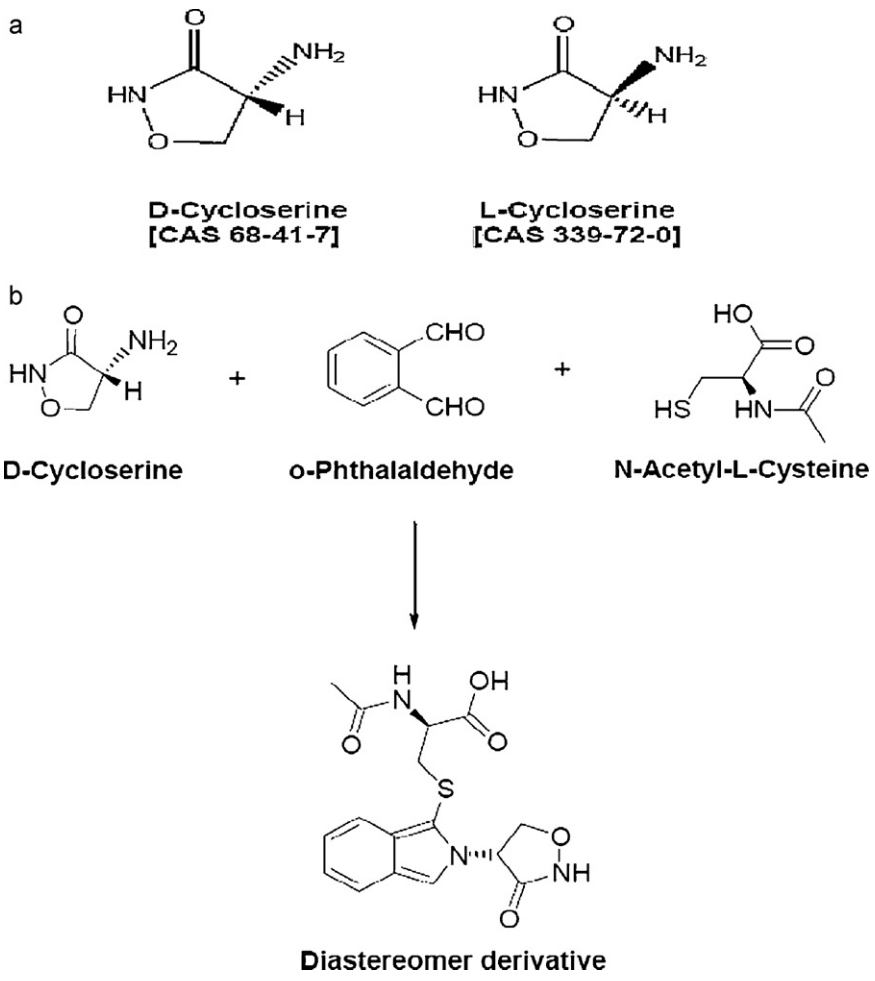

Fig. 1. (a) Chemical structure of D-cycloserine and L-cycloserine. (b) Schematic representation of the formation of diastereomer derivative and its chemical structure.

Millipore (Bedford, MA, USA). Analytical grade boric acid and sodium hydroxide were from Qualigens Fine Chemicals (Mumbai, India) and Ranbaxy Fine Chemicals (New Delhi, India), respectively. Absolute alcohol (AR quality) was from Hayman Limited (Essex, England). o-Phthalaldehyde (purity ≥99.6%) and L-cycloserine (purity ≥95%) were purchased from Sigma–Aldrich (Buchs, Switzerland). N-acetyl-L-cysteine (purity ≥99%) was obtained from Sigma–Aldrich (St. Louis, MO, USA). D-Cycloserine drug substance was obtained from Shasun Chemicals and Drugs Ltd. (Chennai, India).

2.2. Equipments and chromatographic conditions

The HPLC system consisted of a Waters Alliance separation module 2695 equipped with Waters 2487 dual wavelength absorbance detector (Milford, MA, USA). Waters Empower 2 software (Build 2154, Waters) was used for the data acquisition and processing. A Shimadzu LC-2010A HPLC system equipped with UV–VIS detector and having Class-VP software was used for intermediate precision and also for method development purpose (Kyoto, Japan). Peak purity/homogeneity studies were carried out using Waters 2996 photodiode array (PDA) detector (Milford, MA, USA) and Thermo Finnigan Surveyor LC system coupled with LCQ DECA XP Plus ion-trap mass (LC–MS) spectrometer (San Jose, CA, USA). Agilent Zorbax SB phenyl HPLC column of 250 mm length × 4.6 mm id, 5 μm particle size (Palo Alto, CA, USA) was used. The column was kept at (30 ± 2) °C. A mobile phase of 95:05(v/v) 20 mM Na_2HPO_4 (pH 7 using *ortho*-phosphoric acid), and acetonitrile, respectively, was used. Chromatograms were obtained with ultraviolet detection at the wavelength of 335 nm. The injection volume was 15 μL and the flow rate was 1.0 mL/min. The total run time was 20 min.

2.3. Solution preparation

Chiral derivatization reagent (CDR) was prepared by mixing 25.0 mL each of 0.05 M OPA (prepared in ethanol) and 0.05 M NAC (prepared in water) in a 250 mL volumetric flask and diluted to volume with 0.1 M borate buffer (pH 9.5). 0.1 M borate buffer (pH 9.5) was used as diluent in all the preparations. System suitability solution was prepared by dissolving 5.0 mg each of L-cys and D-cys using 5.0 mL diluent in 10 mL volumetric flask and diluted to volume with diluent. 2.0 mL of this solution and 10.0 mL of CDR were added into a 25 mL volumetric flask and diluted to volume using diluent. Resolution between L-cys and D-cys was evaluated as part of system suitability with the acceptance criteria of not less than 1.5. Standard stock was made by dissolving L-cys in diluent to obtain 0.002 mg/mL solution. Standard preparation was made by mixing 3.0 mL of standard stock solution and 10.0 mL of CDR in a 25 mL volumetric flask and diluted to volume using diluent (0.24 μg/mL). Sample preparation was made by dissolving 25 mg of D-cys sample using diluent in 25 mL volumetric flask and diluted to volume. 4.0 mL of this solution and 10.0 mL of CDR were added into a 25 mL volumetric flask and diluted to volume using diluent (0.16 mg/mL).