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Isolation and identification of four major impurities in capreomycin sulfate

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ABSTRACT

Capreomycin has good clinical utility to treat multidrug resistant tuberculosis, but it is only used as a second line drug due to its adverse reactions. Literature has demonstrated that the toxicity of capreomycin product is significantly influenced by the impurities in it. Unfortunately, so far, no one impurity in capreomycin has ever been isolated and definitely identified due to its extremely strong basic character and high polarity. An ion-pair method reported in literature can provide separation of capreomycin and its impurities, but it is hard to be used for the preparative purpose. In this study, this ion-pair method was further improved to detect more impurities in capreomycin sulfate substance. Besides the four main components (IA, IB, IIA and IIB), four impurities (impurity A–D) with their contents much higher than the identification threshold were observed. Furthermore, a two dimensional (2D) LC quadrupole-time of flight (Q-TOF) MS method was established to realize high resolution MS analysis of these impurities. For the purpose of preparative isolation, a hydrophilic interaction chromatography (HILIC) method was established. The four main components were well isolated, but unfortunately, the four impurities were co-eluted with each other or with IB by the HILIC method. Fortunately, the degradation experiments revealed that IA and IB could yield clean impurity A and B respectively in acidic medium, and can yield clean impurity D and C respectively in alkaline medium. Therefore, IA and IB were first isolated by the preparative HILIC method, then pure IA and IB underwent acid degradation and base degradation separately and followed by re-isolation by the HILIC method to obtain pure impurity A–D respectively. Based on Q-TOF MS and NMR analysis, the structures (including absolute configuration) of the four isolated impurities were definitely identified.

1. Introduction

Tuberculosis is an infectious bacterial disease considered as a major cause of illness and death world-wide, especially in developing countries [1,2]. What's worse, tuberculosis is perhaps the most persistent human disease of bacterial origin as increasing drug-resistant or even multidrug resistant strains is being isolated [3,4]. Capreomycin, commonly grouped with the aminoglycosides, was first produced from streptomyces capreolus in the 1960s [5]. Capreomycin has been on the world Health Organization's (WHO) list of essential drugs to treat multi drug resistant tuberculosis [6]. Fattorini et al. demonstrated that only 10% of 46 drug resistant strains of *M. avium* isolated from Italian patients were resistant to capreomycin [7]. However, capreomycin is considered as a second line drug due to its serious undesirable effects, such as renal and hepatic damage, hearing loss and allergic reaction [8]. Lee et al. compared acute toxicity and pharmacokinetic profiles between two drug substances of capreomycin with different impurity contents [9]. The drug substance with fewer impurities was demonstrated to provide much lower toxicity than the other one.

Capreomycin is a mixture of four isoforms: IA, IB, IIA and IIB, which are distributed in the approximate percentages of 25%, 67%, 3% and 6%, respectively [10]. As shown in Fig. 1, the capreomycin molecule is made of a cyclic pentapeptide containing two 2,3-diaminopropionic acids, one β-ureidodehydroalanine, one L-capreomycidine, one serine (for IA and IIA) or alanine (for IB and

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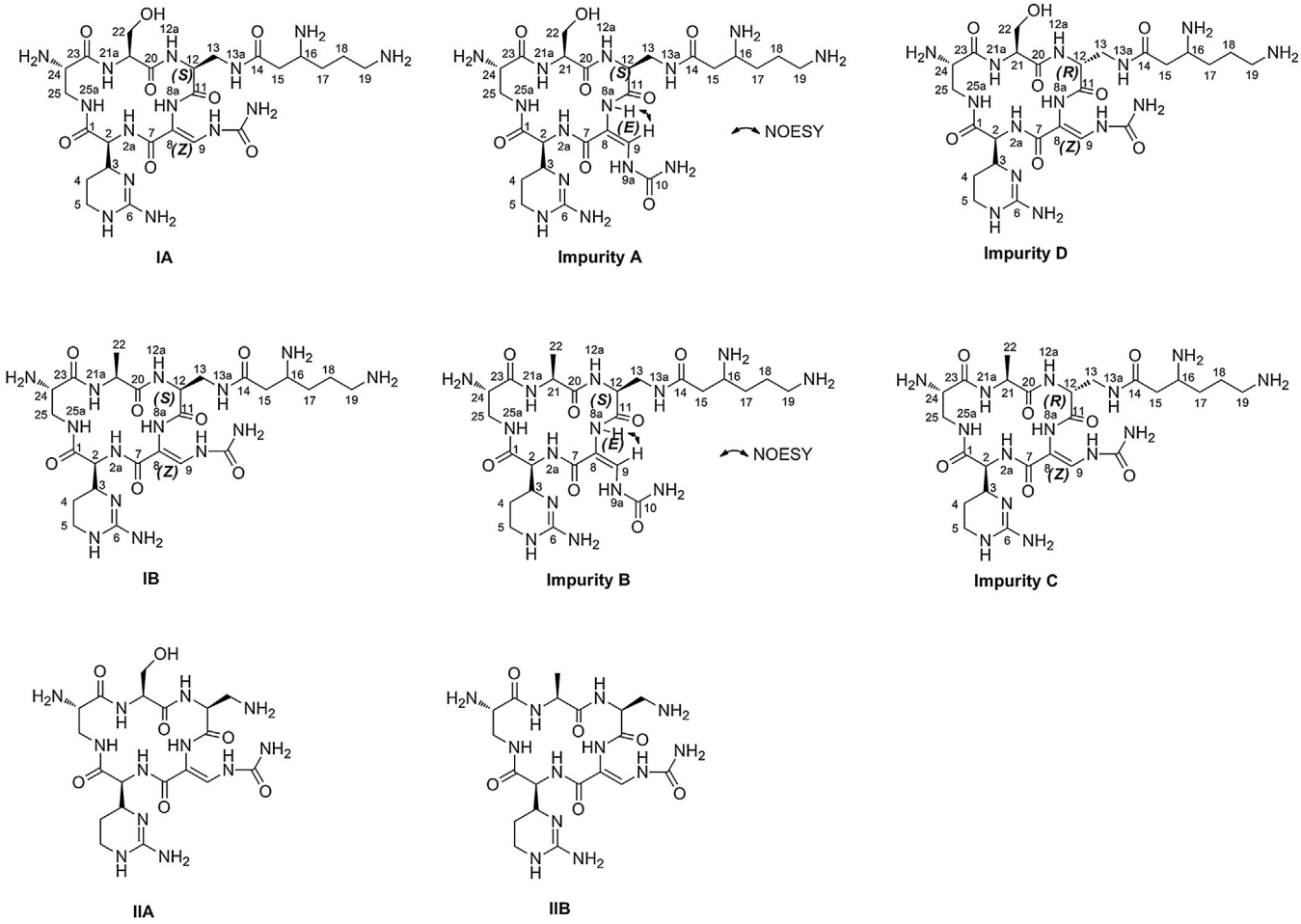

Fig. 1. Structures of the four components and the four major impurities in capreomycin, the NOE correlations supporting the E-configuration of impurity D and C are given.

IIB) and a side chain of β-lysine (only for IA and IB), which are characterized with high polarity and strong basic character. For example, the cyclic guanidine presented on the L-capreomycidine moity is one of the strongest organic bases (pKa ≈ 14) known in chemistry [11]. Therefore, separation of the components and impurities in capreomycin based on chromatography technologies should be a challenging work. For the determination of the capreomycin content, a microbiological turbidimetric assay and a normal phase liquid chromatographic method have been recommended in the British Pharmacopoeia (BP) [12] and the United States Pharmacopeia (USP) [13]. A reverse phase liquid chromatographic method (RP-LC) with ultraviolet detection (UV), by using monopotassium phosphate and a chaotropic reagent of heptafluorobutyric acid was proposed as an alternative method for quantitative analysis of capreomycin in liposome formulations by Rossi et al. [14]. This RP-LC method has been demonstrated to provide enhanced sensitivity and increased resolution with respect to the official method. A hydrophilic interaction chromatography (HILIC) method to quantify capreomycin oleate in a drug formulation for inhalation has also been reported in recent year [15]. Furthermore, a CE method has been reported for the simultaneous determination of capreomycin, ofloxacin and pasiniazide in urine [16]. However, these chromatography methods only have the ability to determine the two major components of IA and IB, the two minor ones, IIA and IIB, and the impurities are hard to separate.

For determination of the impurities in capreomycin, Lee et al. have proposed a thin layer chromatography (TLC) method [9]. The four components and several impurities could be separated. However, the absolute concentration and the identity of impurities in the tested sample are unknown by this simple TLC method. An ion-pair RP-LC method by using phosphate and sodium 1-hexanesulfonate was proposed by Mallampati et al. [17]. Good separation of the four active components of capreomycin and eleven unknown impurities were achieved, but no impurity was identified. Based on this ion-pair RP-LC method, mass spectrometry (MS) analysis of the impurity in capreomycin was realized by Shruti Chopra et al. [18]. Fourteen unknown peaks were detected and collected and desalted and then injected for MS analysis by using a dual liquid chromatography system coupled with an ion trap MS. The fragmentation patterns of the main components were studied, and then the structures of the unknown impurities were deduced by a comparative analysis strategy. However, NMR or other spectroscopic data are needed for further confirmation of those impurities. To the best of our knowledge, no impurity in capreomycin has ever been isolated and definitely identified.

The aim of the present study is to realize preparative isolation and structure elucidation of the impurities in capreomycin substance above 0.15% level (the identification threshold suggested by European Medicines Agency (EMA) for related substances in antibiotics produced by fermentation [19]). The ion-pair method mentioned above can provide good separation, but the ion-pair salt will remain in the collected fraction and is hard to remove via the common post-treatment technology. HILIC has been widely used for separation of hydrophilic and basic compounds due to the ion exchange and/or hydrophobic interaction mechanism [20–22]. Various HILIC phases with different selectivity, such as diol, bare sil-

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