Journal of Pharmaceutical and Biomedical Analysis 120 (2016) 402–412

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Chiral separation of tedizolid using charge single isomer derivatives of cyclodextrins by capillary electrokinetic chromatography☆

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ARTICLE INFO

Article history:
Received 28 August 2015
Received in revised form 6 November 2015
Accepted 15 November 2015
Available online 24 December 2015

Keywords:
Antibacterial agent
CD-cEKC
Cyclodextrin
Enantioseparation
Synthesis
Tedizolid

ABSTRACT

A method to enantioseparate tedizolid (TED), the second analogue after linezolid (LIN) in a truly new class of antibacterial agents, the oxazolidinones, was developed based on capillary electrokinetic chromatography using cyclodextrin as chiral pseudophase (CD-cEKC). The single isomer *R*-tedizolid possesses one chiral centre at C5 of the oxazolidinone ring, which is associated with the antibacterial activity of the drug. Tedizolid enantiomers are non-charged and therefore require the use of charged cyclodextrins (CCDs) as carrier hosts to achieve a velocity difference during migration. During method development, hydrophilic anionic single-isomer and moderately hydrophobic and hydrophobic cyclodextrins were tested, including heptakis-(2,3-dihydroxy-6-sulfo)-β-cyclodextrin (HS-β-CD), heptakis-(2,3-diacetyl-6-sulfo)-β-cyclodextrin (HDAS-β-CD), oktakis-(2,3-diacetyl-6-sulfo)-γ-cyclodextrin (ODAS-γ-CD) and heptakis-(2,3-dimethyl-6-sulfo)-β-cyclodextrin (HDMS-β-CD). Only CDs that have acetyl groups at the C2 and C3 positions with seven (HDAS-β-CD) or eight (ODAS-γ-CD) residues of glucopyranose units provided baseline separation of the tedizolid enantiomers with the addition of organic solvent. During the experiments, different organic solvents were tested, such as methanol, acetonitrile, tetrahydrofuran, which varied in their abilities to donate or accept protons. The best enantiomer separation results were obtained using the CD-cEKC method with 37.5 mM HDAS-β-CD dissolved in 50 mM formic buffer (pH 4.0) with the addition of acetonitrile (81.4:18.6, v/v) at 27 °C, normal polarity, and 12 kV. Finally, the apparent binding constants for each enantiomer—HDAS-β-CD pair were calculated. Moreover, in order to evaluate the behaviour of TED and LIN enantiomers relative to chiral selector, enantioselective interactions towards the precursors of TED and LIN isomers were also investigated.

1. Introduction

Tedizolid ((5*R*)-3-{3-fluoro-4-[6-(2-methyl-2H-1,2,3,4-tetrazol-5-yl)pyridin-3-yl]phenyl}-5-(hydroxymethyl)-1,3-oxazolidin-2-one; Fig. 1A) is a new parenteral antibacterial agent from the oxazolidinone class, which was approved by the FDA in June 2014 and positively endorsed by the CHMP (Committee for Medicinal Products for Human Use) in January 2015. Tedizolid phosphate (proposed trade name SIVEXTRO®) has a favourable safety profile and has been approved to treat acute bacterial skin and skin structure infections (ABSSSI) caused by susceptible bacteria in adults. The spectrum of tedizolid activity includes the following Gram-positive microorganisms: *Staphylococcus aureus* (including methicillin-resistant [MRSA] and methicillin-susceptible [MSSA]), *Staphylococcus haemolyticus*, *Staphylococcus lugdunensis*, *Streptococcus pyogenes, Streptococcus agalactiae*, *Streptococcus anginosus* Group and *Enterococcus faecalis*. Most importantly, tedizolid is active against strains possessing the *cfr* methyltransferase gene responsible for staphylococcal resistance to linezolid [1].

Tedizolid [2] contains a biaryl ring system, the pyridylphenyl oxazolidinone congener. The structure–activity relationships (SARs) of the variations at the C5 position on the oxazolidinone ring revealed unexpectedly strong activity of the hydroxyl group compared with its predecessor linezolid (Fig. 1B), which possesses an acetamide group at the C5 position of the oxazolidinone nucleus [3]. Additionally, the enhanced potency of this molecule against linezolid-resistant strains has been achieved through optimisation of the C- and D-ring systems, pyridine and tetrazole rings, which play critical roles in its activity by forming additional binding interactions with the upper region of the peptidyl transferase centre of

☆ Selected paper from conference of Recent Development in Pharmaceutical Analysis (RDPA2015), June 28-July 1, 2015, Perugia, Italy.

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http://dx.doi.org/10.1016/j.jpba.2015.11.022
0731-7085/

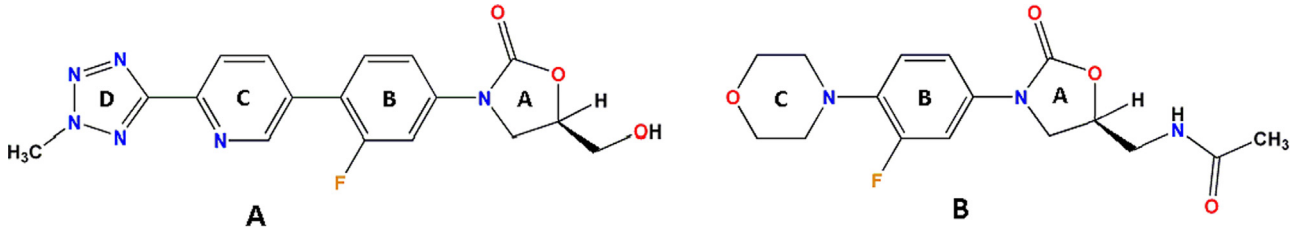

Fig. 1. Molecular structures of tedizolid (A) and its predecessor linezolid (B).

the 50 S ribosomal subunit. The single isomer *R*-tedizolid possesses one chiral centre at the C5 position of the oxazolidinone ring, which is associated with the antibacterial activity of the drug, while the *S*-enantiomer is devoid of this activity [1].

Optically active medicinal products are usually produced as racemates or mixtures of the two enantiomers due to the high cost and technical difficulty associated with their asymmetric synthesis. However, since the report of thalidomide [4], researchers have gradually realized that often only one of the enantiomers has the desired therapeutic activity and favourable pharmacological profile (eutomer), while the second (distomer) is inactive and may contribute to greater toxicity. Therefore, the development of chiral separation methods is crucial for ensuring the quality, safety and efficacy of drugs.

Many review papers and special issues on enantioseparations provide a historical overview of chiral recognition processes, highlight the significance of these research and summarize the most important theoretical principles [5–9]. From the "*three-point attachment model*" to the different thermodynamic aspects in relation to chiral selector—selectand interactions, fundamental aspects of enantioseparations have been achieved using capillary electrophoretic (CE) and liquid chromatographic techniques.

The most popular analytical techniques to separate enantiomers are gas chromatography and high performance-liquid chromatography (HPLC). However, undoubtedly the most promising analytical technique is CE, which is an attractive enantioseparation technique mainly due to: (i) high efficiency, which allows observation of small stereochemical effects of chiral selector - selectand interactions that are invisible to other techniques, (ii) enantioseparation can be obtained in the absence of a binding constant difference between the enantiomers and a chiral selector, as demonstrated by Chankvetadze and Fanali [10], (iii) enantiomer migration order can be adjusted without reversing the affinity pattern between the selector and selectand, (iv) a high degree of flexibility in regards to the optimization of enantioseparation, (v) a short analysis times as well as minimum sample and running buffer volumes, which make it possible to use very expensive chiral selectors, (vi) the applicability of the dual chiral separation system [11], (vii) and non-aqueous capillary electrophoresis (NACE) [12], what significantly increases the versatility of the method development. Enantioseparation may be achieved by adding different chiral selectors, such as cyclodextrins (CDs) [native or derivatives (neutral and/or charged)], metal chelating agents, crown ethers, natural and synthetic chiral micelles, oligo- and poly-saccharides, proteins and antibiotics to the background electrolyte (BGE). Among different chiral selectors, CDs [13] and their derivatives are the most widely employed in chiral CE techniques.

The type and concentration of CD is important to achieve proper separation. Native and neutral CD derivatives are effective chiral selectors for charged enantiomers, but it is impossible to separate neutral enantiomers because they do not possess their own electrophoretic mobility [14].

Charged cyclodextrins (CCDs) were applied as chiral selectors in CE for the first time by Terabe [15]. Besides randomly substituted, charged CD derivatives, a family of single isomer β- and γ-CD derivatives has been synthesized and introduced for the first time by the Vigh's group [16–19]. These researchers synthesized derivatives that are completely sulfated at the C6 positions of glucopyranose units and carried at the C2 and C3 positions, the hydrophilic (hydroxy), moderately hydrophobic (acetyl) and hydrophobic (methyl) functional groups. More recently an analogue carrying non-identical substituents at the C2 and C3 positions have been also synthesized [20]. Especially, an anionic single-isomer CCDs have drawn increase attention in recent years. Several papers mostly regarding to enantioseparation of basic drugs as model analytes have been published using an anionic CD in aqueous [21–24] and non-aqueous CE [22,23,25–31]. The resolution mechanism, in which CDs are used as chiral selectors, is usually based on inclusion complexation [22,23,32], however, external complex formation with excellent separation of enantiomers is also possible [22–25]. Moreover, the possibility of forming the different complexes, such as inclusion and non-inclusion in aqueous and non-aqueous CE have been also demonstrated in these papers [22,23]. It is worth to highlight that the possibility of using NACE is an interesting alternative to improve chiral recognition performance for chiral samples with non or poor solubility in water as well as provides a more favourable environment for chiral discrimination due to their ability to promote intermolecular interactions [12,30].

The aim of this study was to develop principles of chiral separations of the novel oxazolidinone, tedizolid (TED), using charged single isomer derivatives of CDs by capillary electrokinetic chromatography (cEKC). Chiral separation of TED together with linezolid (LIN) enantiomers was investigated to determine the influence of the revised electrophoretic condition on enantioseparation for both representatives of the oxazolidinone group. Moreover, enantioselective interactions towards the synthetized precursors of TED and LIN isomers were also studied to enhance knowledge about the chiral recognition process of oxazolidinones. The identification of domains in oxazolidinone analogues that are responsible for interacting with chiral selectors will form the basis for understanding the biorecognition process involved in drugs binding to their targets and modulating their functions.

To the best of our knowledge, this is the first study that used an EKC to determine the enantioseparation of TED and its precursor. However, over the past few years, some CE methodology has been reported for the chiral separation of linezolid, the predecessor of TED [21]. The mechanism of chiral recognition by NMR spectrometry and a molecular modelling study for LIN were also performed [32].

2. Materials and methods

2.1. Synthesis procedures

TED and LIN precursors and their enantiomers were synthetized in six steps, as described in the Supplementary information, with slight improvements to the literature methods [2,33,34].