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Interactions of non-charged tadalafil stereoisomers with cyclodextrins: Capillary electrophoresis and nuclear magnetic resonance studies

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ABSTRACT

The single isomer drug *R,R*-tadalafil (Cialis®) contains two chiral centers thus four stereoisomers (*R,R*-, *S,S*-, *S,R*- and *R,S*-tadalafil) exist, however, only the most potent inhibitor, the *R,R*-tadalafil is in clinical use.

In our study, over 20 charged cyclodextrin (CD) derivatives were studied for enantiospecific host–guest type interactions in CD-modified capillary electrophoresis. Tadalafil stereoisomers are non-charged; therefore, their electrophoretic separation poses a challenge. Several candidates of both positively and negatively charged hosts were found to be effective for the enantioseparation. Eight out of the beta derivatives and three of alpha derivatives (including sulfated, sulfoalkylated, carboxyalkylated and amino derivatives) resolved all four stereoisomers partially or completely. Cavity size-dependent absolute enantiomer migration order (EMO) reversals were observed in the case of sulfopropyl-alpha (EMO: *R,S*; *S,R*; *R,R*; *S,S*) and sulfopropyl-beta (*S,S; R,R; S,R; R,S*) derivatives, while substituent-dependent partial EMO reversals were detected for sulfobutyl-ether-alpha (*R,S; S,R; S,S; R,R*) and sulfated-alpha-CD (*R,R; S,S*; *R,S*; *S,R*) selectors. Complexation-induced ^{1}H NMR chemical shift changes reflected that the benzodioxole moiety plays a major role in cavity size-dependent EMO reversal.

Sulfobutyl-ether-alpha-CD was the only selector that provided the desired EMO in which the clinically applied eutomer *R,R*-tadalafil migrates last. Finally, an electrophoretic method applying a background electrolyte (BGE) containing 75 mM Tris–acetic acid buffer (pH 4.75) and 7 mM sulfobutyl-ether-alpha-CD was developed for the baseline resolution of all isomers at 25 °C and +25 kV applied voltage.

1. Introduction

Tadalafil (Fig. 1) chemically known as (6*R*,12a*R*)-6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-methyl-pyrazino [1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione is a cGMP specific phosphodiesterase type 5 (PDE-5) inhibitor possessing enhanced PDE-5/PDE-1 and PDE-5/PDE-6 selectivity. Essential advantage of the drug over other PDE-5 inhibitors like sildenafil and vardenafil, resides in its longer half-life. Tadalafil is approved for the treatment of erectile dysfunction, pulmonary arterial hypertension and benign prostatic hyperplasia in various doses. The molecule contains two chiral centers at C6 and C12a, thus giving rise to four stereoisomers (Fig. 1). The stereochemistry of all four stereoisomers was established using vibrational circular dichroism, electronic circular dichroism and optical rotatory dispersion spectroscopy [1]. Comparing the inhibitory potency of the four stereoisomers, the *R,R* isomer was the most potent PDE-5 inhibitor, the *S,S* enantiomer was inactive; while the *R,S* and *S,R* compounds possessed moderate potency [2]. Besides these pharmacological differences, safety profiles are unavailable for the *S,S*, *R,S* and *S,R* isomers, which entails a potential risk of health damage. As tadalafil is becoming more popular and is widely used for the treatment of erectile dysfunction, counterfeit Cialis [3] and dietary

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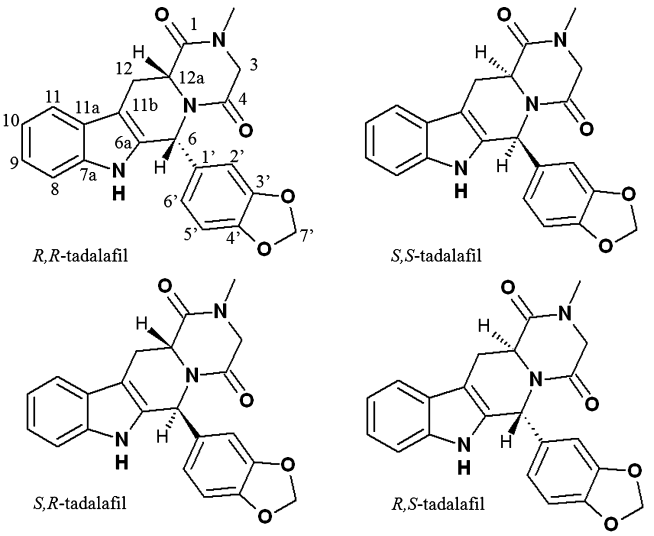

Fig. 1. Chemical structure of *R,R*-tadalafil, *S,S*-tadalafil, *S,R*-tadalafil and *R,S*-tadalafil.

supplements adulterated with tadalafil or its derivatives become an increasing threat. For instance, a diastereomeric mixture of *R,R*-tadalafil and *R,S*-tadalafil (3:1) has been identified in blisters of a counterfeit medicine [3]. Thus, reliable and fast stereoisomeric impurity profiling methods are essential for both the quality control during production of tadalafil and the screening of drug formulations for authenticity. Capillary electrophoresis (CE) has been widely applied in the field of chiral analyses and investigation of the underlying enantioselective inclusion complexation due to its low costs, easy, fast and versatile method development, minute sample and background electrolyte (BGE) need and a wide and easy variation of possible chiral selectors. Furthermore, reversal of the EMO can easily be achieved without modifying the affinity pattern of the selector towards the analyte enantiomers [4–6]. The most commonly used chiral selectors in CE for organic guests are cyclodextrins (CDs). Native α-, β- and γ-CDs consist of six, seven or eight 1-4 linked α-D-glucopyranoside units, respectively, and form a molecule of a truncated cone shape with a hydrophilic exterior surface and a hydrophobic interior cavity enabling the host–guest inclusion complexation. A broad spectrum of CD derivatives is currently available differing in charge, functional groups, degree and position(s) of substitution. In capillary zone electrophoresis (CZE) measurements at least one of the interacting species has to carry a charge. Since tadalafil lacks basic or acidic functional groups ionizable in the pH range of 2–12, separation of its four uncharged stereoisomers requires charged CDs as carrier hosts to afford velocity difference during migration [7–11].

A CE [12] and an HPLC [13] method have been published so far for the determination of *R,R*-tadalafil in bulk samples and an additional HPLC method has been developed for chiral separation of the *R,R* and *S,S* enantiomers [14].

Separation of all four stereoisomers of a drug with two chiral centers has already been accomplished, see e.g. fencamfamine [15]. The published examples include exclusively ionizable (charged) molecules. In the case of tadalafil, separation of all four neutral stereoisomers has to be achieved for the first time, which is a significantly more challenging task by CZE.

Our aim was to investigate the inclusion complexation properties of tadalafil stereoisomers by screening a wide variety of CDs in aqueous CE and to develop a chiral separation method, which may serve as a routine tool for stereoisomeric impurity profiling of tadalafil. To gain more insight into mechanistic aspects of the separation, we also intended to characterize the complex formation with the most promising CDs by ^{1}H NMR spectroscopy. Although NMR offers a variety of methods for this purpose, such as titration, diffusion or structural studies based on nuclear Overhauser enhancements [15,16], the very poor solubility of the substance even in solvent mixtures severely limited the possibilities. Complexation induced ^{1}H NMR chemical shift changes were monitored in the case of sulfopropyl-α and sulfopropyl-β CDs to gain insight into host–guest interactions possibly responsible for EMO reversal.

2. Materials and methods

2.1. Synthetic procedures

The synthetic process of *R,R*-tadalafil and its epimers is available in the literature [2]. *R,R*-Tadalafil was purchased from Sigma-Aldrich, 6-Epi-tadalafil [17] and 12-epi-tadalafil were prepared according to the known procedures [18]. The (6*S*,12a*S*) distomer was synthesized in three steps as described in the supplementary information, with slight improvements of the literature process [2].

2.2. Materials

All CD derivatives with various degrees of substitution: carboxymethylated-α-CD DS∼3.5 (CM-α-CD), carboxyethylated-α-CD DS∼3 (CE-α-CD), sulfated-α-CD DS∼9 (S-α-CD), sulfopropylated-α-CD DS∼2 (SP-α-CD), sulfobutyl-ether-α-CD DS∼4 (SBE-α-CD), carboxymethylated-β-CD DS∼3 (CM-β-CD), carboxyethylated-β-CD DS∼3 (CE-β-CD), succinylated-β-CD DS∼6 (Succ-β-CD), phosphated-β-CD DS∼4 (Phos-β-CD), sulfated-β-CD DS∼11 (S-β-CD), sulfopropylated-β-CD DS∼4 (SP-β-CD), sulfohydroxypropylated-β-CD DS∼2.5 (SP-β-CD), sulfobutyl-ether-β-CD DS∼4 and DS∼6.3 (SBE-β-CD), 6-monodezoxy-6-monoamino-β-CD DS∼1 (MA-β-CD), 6-monodezoxy-6-mono(3-hydroxy)propylamino-β-CD DS∼1 (PA-β-CD), 6-monodezoxy-6-mono(2-hydroxy)proplyamino-β-CD DS∼1 (IPA-β-CD), carboxymethylated-γ-CD DS∼4 (CM-γ-CD), sulfated-γ-CD DS∼11.5 (S-γ-CD), sulfopropylated-γ-CD DS∼2 (SP-γ-CD), sulfohydroxypropylated-γ-CD DS∼3 (SHP-γ-CD), sulfobutyl-ether-γ-CD DS∼4 (SBE-γ-CD) were products of Cyclolab Ltd. (Budapest, Hungary). Sodium acetate, acetic acid, HCl, NaOH, Tris, NaH_2PO_4, Na_2HPO_4 and boric acid used for the preparation of buffer solutions, methanol and acetonitrile, applied as organic modifiers were of analytical grade and purchased from commercial suppliers. D_2O (99.9% D atom) and CD_3OD (99.8% D atom) were products of Merck KGaA (Darmstadt, Germany). All reagents were used without further purification. Bidistilled Millipore water was used throughout this study.

2.3. Capillary electrophoresis

All CE experiments were carried out on an Agilent 7100 Capillary Electrophoresis instrument (Agilent Technologies, Waldbronn, Germany), equipped with a photodiode array detector and the Chemstation software for data handling. Untreated fused silica capillaries (50 μm id, 48.5 cm total and 40 cm effective length) were purchased from Agilent. Conditioning of new capillaries was conducted by flushing with 1 M NaOH followed by 0.1 M NaOH and water for 30 min each. As the first and last conditioning the capillary was rinsed every day with 1 M NaOH for 5 min followed by 0.1 M NaOH and water for 15 and 10 min, respectively. Prior to all runs, a preconditioning of rinsing with 0.1 M NaOH (3 min), water (3 min) and BGE (5 min) was conducted before further optimization. The temperature of capillary was set to 25 C. During the measurements 25 kV voltage was applied in normal polarity mode, UV detection was performed at 200 nm, samples were injected hydrodynamically (20 mbar 3 s) and run in triplicate. The running buffers were 25 or 75 mM Tris adjusted to pH 4.75 by adding glacial acetic acid;