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Enantiomeric separation of tapentadol by capillary electrophoresis—Study of chiral selectivity manipulation by various types of cyclodextrins

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ABSTRACT

The chiral recognition of the centrally acting analgesic agent tapentadol and its isomers with various cyclodextrins (CDs) was studied by capillary electrophoresis, focusing on the migration order of four stereoisomers. In the case of non-charged hydroxypropylated CDs (2-hydroxypropyl-β-CD, 2-hydroxypropyl-γ-CD) the beta derivative was able to discriminate the *S,R*- and *R,S*-isomers in acidic background electrolyte, whereas the gamma allowed the separation of *S,S*- and *R,R*-tapentadol, respectively. Dual CD system containing both hosts was used to separate all of four isomers. Negatively charged sulfated-α-CD at 1.0% (w/v) concentration in 100 mM sodium borate buffer (pH 9.5) was capable of separating the isomers with favorable enantiomer migration order and the optimized method was able to determine 0.15% of chiral impurities of tapentadol in the presence of the last migrating clinically important *R,R*-isomer.

1. Introduction

Tapentadol [(*2R,3R*)-3-(3-hydroxyphenyl)-*N,N*-2-trimethylpentan-1-aminium chloride, $C_{14}H_{24}NO^{+}\cdot Cl^{-}$], **Nucynta®** is a novel centrally acting drug that combines μ-opioid receptor agonism and noradrenaline reuptake inhibition, producing analgesic effects in various painful conditions [1]. Tapentadol has two chiral centers leading to four possible isomers. The X-ray crystal structure analysis reveals its stereochemistry at the 3-ethyl- and 2-methyl-groups. The chemical structure of tapentadol indicating the chiral centers is presented in Fig. 1. Only the *R,R*-isomer is in clinical use [2].

As the control of enantiomeric contamination is required by Pharmacopoeias and regulatory agencies, for routine analysis it is necessary to have high throughput, low cost analytical method with simple sample preparation and small sample consumption. Capillary electrophoresis (CE) easily fulfills the above mentioned requirements. Comparing to High Performance Liquid Chromatography (HPLC), CE utilizes the wide spectrum of chiral selectors (e.g. native and derivatized cyclodextrins, crown ethers, antibiotics, polymers) as buffer additives for chiral recognition without the need for expensive columns [3,4]. Moreover, CE mostly provides shorter analysis time and better resolution and CE offers the advantage of altering the enantiomer migration order (EMO) based on the change of the type of chiral selector [5–7].

The most commonly used chiral selectors are the cyclodextrins (CDs) due to their wide variety of cavity size, side chain, degree of substitution (DS) and charge. Small changes in the CD structure, substitution pattern or cavity size could lead to different affinity toward the isomers of the analyte and frequently enabling the alteration of the EMO [5]. The manipulation of EMO plays an important role in the determination of minor components (often as chiral impurities), as peak shape distortion of the major compound (tailing or fronting) can deteriorate the detection and quantitation of the minor components. It is generally accepted

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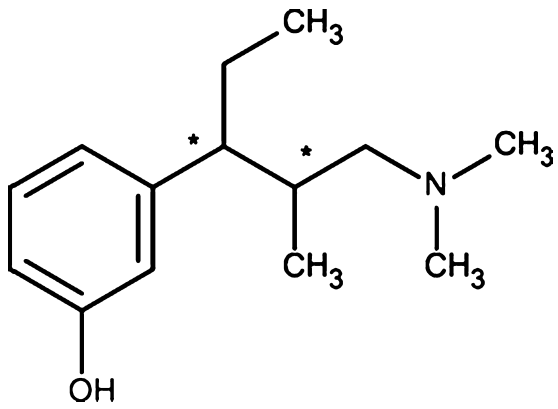

Fig. 1. Chemical structure of tapentadol. Chiral centers are indicated by asterisks.

that the enantioseparation in CE is usually based on the different binding affinity of the enantiomers to the chiral selector. However, it has also been proved that the mobility differences between the temporary diastereomeric associates formed between the enantiomers and the chiral selector can result in enantioseparation even in the case of non-enantioselective binding [5,8].

There is a single polysaccharide derivative-based liquid chromatographic method in the literature for the separation of all four isomers of tapentadol [9], while our preliminary CD-based CE method [10] deals with the enantioseparation of the *S,S*- and the *R,R*-enantiomer pair of tapentadol only.

Our present study provides various enantiorecognition of all four tapentadol stereoisomers by CD-modified CE with the potential of changing the EMO. In this paper the correlations between the chiral selectivity, the enantiomer migration order, the apparent, averaged tapentadol-selector complex stability constants and the complex mobilities are discussed. The proposed method was partially validated and tested on a real sample.

2. Materials and methods

2.1. Apparatus

The CE experiments were performed on a HP ^{3D}CE and on an Agilent 7100 instrument (Agilent Technologies, Waldbronn, Germany) equipped with a photodiode array detector (DAD). Uncoated fused silica capillary was used (MicroSolv, 50 μm i.d., 365 μm o.d., total capillary length 64.5 cm, effective capillary length 56.0 cm for the preliminary experiments and for final conditions, whereas 48.5/40 cm capillary was applied for the determination of the apparent complex stability constants and 33.5/25 cm for the dual system conditions). The capillary was thermostated at 15 °C; the separation voltage was set to ±25 kV according to the given background electrolyte (BGE) system. Samples were injected hydrodynamically (25 mbar for 5 s) with normal-end injection as well as with the short-end injection (8.5 cm effective length to the detector) at acidic pHs. The capillary was washed daily before the experiments and after each BGE change with 0.1 M sodium hydroxide for 20 min and deionized water for 15 min. Between analyses the capillary was washed with proper BGE containing the chiral selector for 3 min.

2.2. Chemicals

Tapentadol standards (*R,R*-, *R,S*-, *S,R*-, *S,S*-tapentadol) were kindly provided by Zentiva Group, a.s. (Prague, Czech Republic). Boric acid, phosphoric acid, Tris, sodium hydroxide, lithium hydroxide were purchased from Sigma (St. Louis, MO, USA). Native CDs (α-, β-, γ-), non-charged hydroxyethyl-β-CD (HE-β-CD) average degree of substitution (DS ~ 3), (2-hydroxypropyl)-γ-CD (2-HP-γ-CD), (2-hydroxypropyl)-β-CD (2-HP-β-CD) (DS ~ 0.5–1.3), negatively charged sulfobuthyl-ether-α-CD (SBE-α-CD), sulfated CDs (S-α-CD (DS ~ 8.7), S-β-CD (DS ~ 11), S-γ-CD (DS ~ 11.5)), carboxymethylated-α-CD (CM-α-CD) (DS ~ 3.5) and carboxymethylated-β-CD (CM-β-CD) (DS ~ 3) and carboxyethylated-β-CD (CE-β-CD) were purchased from Sigma (St. Louis, MO, USA).

BGEs were prepared by dissolution of the appropriate amounts of boric acid or phosphoric acid in deionized water (18 MΩ cm^{-1}) and pH was adjusted by Tris, sodium hydroxide or lithium hydroxide to the desired values (2.0–10.5). Then the chiral selector was added individually.

The standard solutions of each enantiomer were prepared separately in 1 mg mL^{-1} concentrations by dissolution of the standard compound in deionized water. The racemic mixture was prepared by mixing of all four enantiomers. The working solutions were prepared by dissolution of stock solutions and were stored at −20 °C. All measurements were repeated five times. The individual isomers were identified by spiking the analyte with known concentration of standard solution.

3. Results and discussion

3.1. Preliminary experiments

Tapentadol, as a diprotic compound has pK_a values of 9.44 and 10.59 at 25.0 °C and 0.15 M ionic strength confirmed by ^{1}H NMR-pH titration [10]. Due to the protonation of the amino group, tapentadol is positively charged under acidic conditions (e.g. in phosphate BGE at pH 2.5) and partially positive under basic BGE conditions (in borate buffer at pH 9.5).

In preliminary experiments, various native (α-, β-, γ-CDs), non-charged (HE-β-CD, 2-HP-γ-CD, 2-HP-β-CD) and negatively charged (SBE-α-CD, S-α-CD, S-β-CD, S-γ-CD, CE-β-CD, CM-α-CD and CM-β-CD) CDs were used for screening of enantioseparation of all four enantiomers. The chiral recognition was tested in acidic (phosphate pH 2.5) and basic (borate pH 9.5) BGEs at various CD concentrations ranging from 0.1 to 5.0% (w/v). Under acidic conditions the effect of EOF was minimized due to negligible ionization of silanol groups on the inner capillary wall. To shorten the analysis time at pH 2.5 short end injection (8.5 cm effective length) was conducted.

3.2. Screening with native- and non-charged CDs

The effect of all three native CDs (α-, β-, γ-CD) was studied in 50 mM sodium phosphate (pH 2.5) and 50 mM sodium borate (pH 9.5) BGE. The α-CD at alkaline conditions was able to separate *R,R*-, *S,S*- and *R,S*-, *S,R*-tapentadol isomers at concentration of 1.0% (w/v) however with poor peak efficiency. The β-CD at 0.1% (w/v) allowed the separation of *RR-/SS*- and *SR-/RS*- diastereomeric pairs and partial enantioseparation of the *R,S*-isomer from *S,R*-isomer under acidic conditions. With increasing β-CD concentration the enantioresolution decreased. In the case of γ-CD, only the *RR-/SS*- and *SR-/RS*-diastereomeric pairs were separated at a concentration of 0.5% (w/v) in acidic BGE. For the accurate quantification of an individual enantiomer achieving higher resolution than 1.5 is necessary, thus various substituted CD derivatives were studied in the next step.

The non-charged HE-β-CD, 2-HP-γ-CD and 2-HP-β-CD selectors were also subjected to screening. The separation applying HE-β-CD resulted in baseline separation for *R,S*- and *S,R*-pair of isomers at both pH, whereas the *S,S*- and *R,R*-isomers did not separate at all. The hydroxypropylated CDs (2-HP-β-CD and 2-HP-γ-CD)