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Using single-isomer octa(6-O-sulfo)-γ-cyclodextrin for fast capillary zone electrophoretic enantioseparation of pindolol: Determination of complexation constants, software-assisted optimization, and method validation☆

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ABSTRACT

The present study describes a rapid and effective capillary electrophoresis (CE) method for the enantioseparation of pindolol using single-isomer octa(6-O-sulfo)-γ-cyclodextrin. The complexation parameters were determined under neutral and high pH conditions to identify optimal separation conditions using a theoretical model. Baseline separation of pindolol enantiomers was achieved within 6 min in a sodium/MOPS buffer, pH 7.2, with a selector concentration of 6 mM. The method was validated according to the ICH guidelines using imidazole as an internal standard. Low limits of detection and quantification were found, specifically 1.2 μg/mL and 4 μg/mL (0.6 μg/mL and 2 μg/mL per enantiomer), respectively. The calibration curves showed good linearity, with a coefficient of determination $R^2 \geq 0.999$ over a 5 – 55 μg/mL concentration range and over a 50 – 300 μg/mL concentration range of the racemic mixture. The relative standard deviations (%RSD) of intra-day and inter-day precision were lower than 8% at LOQ level, lower than 3% at 50 μg/mL level and lower than 1.5% at 300 μg/mL level. Accuracy ranged from 95 to 103% (106% at LOQ level). The proposed method was successfully tested on a medical formulation of Visken® Sandoz intravenous solution and Visken® Teofarma pills for oral use.

1. Introduction

Pindolol belongs to the group of β-blockers, clinically important drugs, which block β-adrenergic receptors. Because pindolol is a nonselective β-blocker, it interacts with both β1- and β2-receptors. Similarly to many other drugs, pindolol is a chiral compound with a chiral center and two enantiomers. Although the racemic mixture is used in clinical practice, the biological activity of the R-(+) enantiomer is approximately 200 times lower than that of the S-(−) enantiomer [1]. This drug is mainly used to treat various cardiovascular disorders, *e.g.*, angina pectoris, cardiac arrhythmia or hypertension [2,3]. Some studies have shown that pindolol is useful in the treatment of glaucoma [4,5], and successes have been also achieved in the treatment of mental disorders such as schizophrenia and panic disorders [6,7]. Although enantiomeric purity is not always a requirement for successful treatment, chiral separations of β-blockers enable the pharmacokinetics and pharmacodynamics study of both enantiomers. Furthermore, only the therapeutically active enantiomer of medical drugs should be available in the market or, when this is not possible, the other enantiomer should be treated as other impurities in the formulation, according to the European Medicines Agency [8], which increases the significance of chiral separations in general.

In addition to chromatography [9–14], enantioseparation of β-blockers has recently stood out in capillary electrophoresis (CE). Several review papers focused on this topic can be found in the literature [14–21]. Among the various chiral selectors that can be used for this purpose, cyclodextrins (CDs) have remained the most popular chiral recognition agents in CE since the early 1990s [22,23]. Derivatization of the native CD ring extends the separation potency of CDs and provides new physicochemical properties, such as increased solubility in aqueous solution, acidic/basic properties or a permanent charge, to these oligosaccharides. Simple derivatization usually results in a mixture of isomers that differ in both the number and the positions of the substituents. This is caused by the symmetry of the CD molecule, which consists of 6, 7 or 8 (α-, β- or γ- CDs, respectively) equivalent α-D-glucopyranoside units.

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Although we have shown that such mixtures of isomers can be advantageous for chiral resolution due to the so-called mixed thermodynamic/electrophoretic enantioseparative mechanisms [24], batch-to-batch reproducibility remains an issue of randomly substituted mixtures of CDs.

To overcome this issue, synthetic strategies have been developed for single-isomer CD derivatives. Since Vigh and coworkers [25] reported the first member of a family of negatively charged, highly sulfated CDs in 1997 new derivatives have been regularly emerging until the most recent publication in 2017 [26]. The original idea of Vigh and coworkers was based on 1) protecting the primary hydroxyl group of the α-D-glucopyranoside units, 2) protecting the secondary hydroxyl groups, 3) deprotecting the primary hydroxyls and 4) derivatizing the primary hydroxyls in a sufficient excess of the derivatization agent, resulting in a single-isomer CD derivative with all α-D-glucopyranoside primary hydroxyls derivatized. This protocol is apparently cost-effective and easy to follow, and various selectors are commercially available (https://cyclolab.hu/, http://www.cyclodextrin-shop.com/). A review by Chankvetadze focused on using charged cyclodextrins in capillary electrophoresis [27] and another by Cucinotta et al. summarized the use of charged single-isomer CD derivatives in CE [28]. Although the range of applications is wide, the available data on rational method development and validation tests remains scarce.

In this study, we present a new CE method for simultaneous enantioseparation and quantification of pindolol using octa(6-O-sulfo)-γ-cyclodextrin (OS-γ-CD). OS-γ-CD [29,30] belongs to the family of commercially available CD derivatives introduced by Vigh and coworkers. Herein, we show that, although OS-γ-CD is a single isomer, its high separation potency allows the fast and reliable enantioseparation of this drug. The analyte-selector complexation parameters were determined, and their contribution to separation efficacy was analyzed for method optimization. The method was validated and showed appropriate accuracy and precision. The limits of detection and quantification matched those provided by HPLC using the same type of detection [31]. The reliability of this technique was also tested on a real sample of pindolol drug formulations.

Regarding HPLC, pindolol enantioseparations from water solution [32] and from various matrices, such as urine or human serum [33], have been previously performed. Some of these enantioseparations were followed by pharmacokinetics analysis [34,35] and simultaneous quantification of both enantiomers [35,36]. As previously reported [32,36], the use of a UV detector provides limits of detection (LOD) in the order of μg/mL. However, lower LODs can be achieved when incorporating a different type of detector; fluorescence detectors provide LODs in a magnitude of ng/mL [34], albeit requiring prior pindolol derivatization. Even lower LODs can be achieved by MS or by MS/MS detection [37]. CE is sometimes mentioned as a green alternative to HPLC because CE avoids or minimizes the use of organic solvents and requires a low consumption of chemicals in general [38,39]. Pindolol enantiomers have been predominantly separated by CE in phosphate buffer at acidic pH using cyclodextrins [40–42]. In addition to experiments in common buffers, effective separation has also been achieved in systems containing organic modifiers [43,44] and in systems using different selectors, including micelles [45] and clarithromycin [44]. Enantioseparations by CE with sufficient resolution ($R > 1.5$) in times ranging from 12 to 35 min have been reported. However, the OS-γ-CD used in this study is able to baseline-separate the two pindolol enantiomers within 6 min. Furthermore, no validation data on pindolol enantioseparation by CE has been published thus far. Although Briguenti and Bonato [37] validated the quantitative analysis of pindolol and a few other β-blockers in pharmaceutical preparations by capillary electrophoresis, the method was not developed for enantioseparation.

Table 1
Preparation of calibration and validation solutions of pindolol: V μL of pindolol/BGE stock solution (1 mg/mL) mixed with 25 μL of imidazole/BGE stock solution (150 mM) and 25 μL of DMSO/water solution (2% v/v), and completed to the total volume of 5 mL with the BGE, resulting in a c_{tot} concentration of pindolol racemate (per enantiomer in parenthesis) in the solution. Data for low and high concentration ranges.

Lower range						
c_{tot} / (μg/mL)	5 (2.5)	15 (7.5)	25 (12.5)	35 (17.5)	45 (22.5)	55 (27.5)
V / μL	25	75	125	175	225	275
Higher range						
c_{tot} / (μg/mL)	50 (25)	100 (50)	150 (75)	200 (100)	250 (125)	300 (150)
V / μL	250	500	750	1 000	1 250	1 500

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical-grade purity: Racemic pindolol, MOPS (3-(N-morpholino)propanesulphonic acid), MOPS-Na (3-(N-morpholino)propanesulphonic acid sodium salt), imidazole and DMSO (Sigma-Aldrich, Prague, Czech Republic), NaOH solution for rinsing the capillary (Agilent Technologies, Waldbronn, Germany). S-pindolol standard was purchased from Santa Cruz Biotechnology, Inc., Heidelberg, Germany. Chiral selectors octa(6-O-sulfo)-γ-cyclodextrin (OS-γ-CD, M = 2111.9 g/mol) and hepta(6-O-sulfo)-β-cyclodextrin (HS-β-CD, M = 1849.4 g/mol) were generous gifts from prof. Gyula Vigh (Texas A&M university, College Station, TX, USA). Pindolol formulation Visken® Sandoz 0.4 mg/2 mL (Sandoz, Basel, Switzerland) was a generous gift from Dr. Radomír Čabala (Charles University, Prague, Czech Republic) and Visken® Teofarma 5 mg dosage (TEOFARMA Srl, Italy) was a generous gift from Jitka Čáslavská (Universität Bern, Berna, Switzerland). Water was purified by Rowapur and Ultrapur (Watrex, San Francisco, CA, USA). IUPAC buffers of pH 4.005 and 10.012 (Radiometer, Copenhagen, Denmark) were used to calibrate the pH meter.

2.2. Instrumentation

Experiments were performed using an Agilent CE 7100 system with a diode array UV/VIS detector, operated *via* ChemStation software (Agilent Technologies, Waldbronn, Germany) in bare fused-silica capillary, ID 50 μm, OD 375 μm, effective length 40 cm, total length 48.5 cm (Agilent Technologies, Waldbronn, Germany). A PHM 240 pH/ION Meter (Radiometer Analytical, HACH Company, Loveland, CO, USA) was used to measure the pH of the solutions.

2.3. Experimental conditions

Sodium MOPS buffer containing 40 mM Na and 80 mM MOPS (pH 7.12) was used. The stock solution of 6 mM OS-γ-CD in the buffer served as BGE. Calibration solutions and solutions used for the validation study were prepared according to Table 1. The running buffer with the selector was used for sample preparation. All solutions were filtered using 0.45-μm syringe filters (Sigma-Aldrich, Prague, Czech Republic).

All experiments were performed under the following conditions: temperature, 25 °C; voltage, +25 kV; detection wavelength, 214 nm; hydrodynamic injection, 20 mbar × 6 s A new capillary was flushed 5 min with water, 5 min with 0.1 M NaOH and again 5 min with water. The same procedure was applied each day after the measurements. Before the measurements, the capillary was rinsed with BGE for 10 min and for 3 min between each experiment, using