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A novel in situ strategy for the preparation of a β-cyclodextrin/polydopamine-coated capillary column for capillary electrochromatography enantioseparations

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**Abbreviations:** β-CD, β-cyclodextrin; DA, dopamine; PDA, polydopamine; OT, open tubular; SP, streaming potential;

**Keywords:** capillary electrochromatography, chiral separation, cyclodextrin, polydopamine,

**ABSTRACT**

Inspired by the chiral recognition ability of β-cyclodextrin and the natural adhesive properties of polydopamine under alkaline conditions, in this study, a rapid and in situ modification strategy was developed to fabricate β-cyclodextrin/polydopamine composite material coated-capillary columns for open tubular capillary electrochromatography. The results of scanning electron microscopy, FTIR spectroscopy, streaming potential and electro-osmotic flow studies indicated that β-cyclodextrin/polydopamine was successfully fixed on the inner wall of the capillary column. This coating can be achieved within 1 h affording a greatly reduced capillary preparation time. The performance of the β-cyclodextrin/polydopamine-coated capillary was validated by the analysis of seven pairs of chiral analytes, namely epinephrine, norepinephrine, isoprenaline, terbutaline, verapamil, tryptophane, carvedilol. Good enantioseparation efficiencies were achieved for all. For three consecutive runs, the relative standard deviations for the migration times of the analytes for intra-day, inter-day and column-to-column repeatability were in the range of 0.41–1.74%, 1.03–4.18%, and 1.66–8.24%, respectively. Moreover, the separation efficiency of the β-cyclodextrin/polydopamine-coated capillary column did not decrease obviously over 90
runs. The strategy should also be feasible to introduce and immobilize other chiral selectors on the inner walls surface of capillary columns.

1. Introduction

The separation of chiral compounds is becoming increasingly important in many industries, particularly the pharmaceutical industry[1,2]. This interest is due to the different pharmacokinetic characteristics and pharmacological activities of each enantiomer in a racemic drug. Many different types of instrumentation and methods have been utilized for enantioseparation, including HPLC [3], GC [4], and CE [5,6]. Compared to HPLC and GC, CE offers advantages of high separation efficiency, rapid analytical time and low sample volume, Theses have won it acceptance as a technique. Make it particularly appropriate for analytical-scale enantioselective separations[7].

CEC, a hybrid technique of the two separation techniques of CE and HPLC, has attracted increasing attention in recent years due to some interesting properties such as superior selectivity, resolution and separation efficiency. Besides, CEC provides short separation times and a minimal consumption of materials and chemicals[8]. As one type of CEC column, the open tubular (OT) column is promising for separation due to its advantages including ease of preparation (no need for end frits and particles packing), no bubble formation, highly stable stationary phase and the high reproducibility[9].
Various coating techniques have been used to develop OT-CEC columns, including chemical modified covalent coating[8], physically adsorbed coating[10], and dynamic coating[11]. However, the preparation process of the covalently coated capillary is rather complicated and time-consuming which includes multi-steps such as capillary pretreatment, introducing coupling agents, and inserting target coating reagents[12]. Dynamic coating is simple but the coating is less stable because of the dynamic equilibrium existing between coating materials in the solution and those deposited on the surface[13]. As for columns with physically adsorbed coatings, they are easier to fabricate with less cost, and show good stability and separation performance comparable to columns with covalent coating[14].

In most CE and CEC studies, enantioseparation has been carried out with chiral selectors such as cyclodextrins (CDs)[15,16], chiral crown ethers, chiral calixarenes, chiral ligand exchange complexes[17], macrocyclic antibiotics, protein[18], chiral surfactants or chiral metal–organic frameworks[19]. Among these chiral selectors, α-, β-, γ-CDs and their derivatives have been used extensively in chiral separations[20]. The mechanism is based on the inclusion or incorporation of analytes, or at least a part of the analytes, in a hydrophobic basket of the various CDs. Some remaining interactions such as hydrogen bonding, steric interactions, and electrostatic interactions for charged CDs also contribute to chiral recognition[15]. However, CDs often need to be modified into thiolated CDs[21], sulfated CDs[22], carboxymethyl-CDs[23] or azide-CDs[24], so that, during the stationary phase, they will be immobile on the pretreated capillary inner wall. To achieve this goal, the modification
must be precise, and the preparation is both tedious and time consuming. Therefore, a simple strategy for the preparation of CD-modified OT capillary columns would greatly expedite their use in every application.

Inspired by the composition of adhesive proteins in mussels, Messersmith’s group found that dopamine (DA) can self-polymerization to form thin, surface-adherent polydopamine (PDA) films on a wide range of inorganic and organic materials[25]. It is believed that the mechanisms of interaction between polydopamine (PDA) and substrates can be divided into two types principally depending on whether the substrates bind covalently or non-covalently. Although the chemical composition of PDA coating is not exactly understood, it is known that the coating contains catechol and quinine functional groups. It has also been established that the covalent binding occurs with specific substrates that contain amine and/or thiol groups on their surfaces by Michael addition and/or Schiff base reactions under alkaline conditions[26–29]. At the same time, PDA is prone to diffusion on substrates through noncovalent binding interaction such as metal coordination or chelating, hydrogen bonding, π–π stacking, and quinhydrone charge-transfer complexes to yield an effective adhesion layer[30–32]. Since Yin and Liu developed firstly a low cost, easy-to-prepare and efficient OT capillary column with PDA coating for determination of auxins[33], some versatile OT-CEC methods using mussel-inspired PDA-assisted coatings as the stationary phases have been developed[34–36]. In 2011, Shimmo’s group applied PDA as an adhesive for biomaterial-based secondary coatings, to achieve better attachment of lipids, cell membrane
pieces and intact mitochondria to fused-silica capillary walls[37], Their successes opened new fields of application for PDA coating.

Therefore, PDA coating shows promise as a molecular means to attach CDs onto capillary inners wall for chiral separation. In this work, the β-cyclodextrin/polydopamine (β-CD/PDA) composites fixed onto the inner walls of capillary column in one step by filling the capillary with dopamine and β-CD mixed aqueous solution and then leaving it for 1 h at room temperature. This method not only reduces the amount of β-CD, but also presents a simple way to prepare the chiral stationary phase. The performance of the new β-CD/PDA coated OT capillary column was evaluated for its ability to separate chiral drugs and monoamine neurotransmitters.

2. Materials and Methods

2.1. Chemicals and materials

Methanol, ethanol, dimethylsulfoxide (DMSO), and β-CD were obtained from Tianjin Guangfu (Tianjin, China). NaOH, HCl and Tris-HCl were obtained from Chengdu Kelong (Chengdu, China). Na₂B₄O₇·10H₂O was purchased from Xi’an Chemical Plant (Xi’an, China). D,L-tryptophane and dopamine (DA) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Isoprenaline was bought from J&K Scientific (Beijing, China). Terbutaline, norepinephrine and epinephrine were purchased from National Institutes for Food and Drug Control (Beijing, China). Carvedilol and verapamil were extracted with ethanol from
carvedilol tablets and verapamil tablets which were both purchased from local drug store (Lanzhou, China). Chemical structures of the analytes are shown in Fig. S1. Fused-silica capillary (375 µm o.d. × 75 µm i.d.) was purchased from Yongnian Photoconductive Fiber Factory (Hebei, China). Unless otherwise stated, all chemicals and reagents were analytical grade and used without further purification. Redistilled water was used throughout the experiment.

2.2. Apparatus and characterization

FTIR spectra were collected on a Nicolet Nexus 670 FTIR spectrometer (USA) using KBr pellets with DTGS detector in the mid-IR range. SEM images were recorded on a JSM-6701F cold field emission scanning electron microscope. Measurements of the streaming potentials (SPs) of capillaries were carried out on a homemade apparatus equipped with two Pt electrodes and an USB-1208 FS data acquisition card (Norton, USA). A pH meter (pHS-3C, Shanghai Precision & Scientific Instrument, China) equipped with a glass electrode was used to adjust the apparent pH (pH*) values of the running buffers in this work. The electrode was calibrated using standard aqueous buffers, pH 4.00, 6.86 and 9.18 at 25.0 ± 0.2°C.

2.3 Preparation of capillary columns coated with β-cyclodextrin/polydopamine

The coated capillary columns used in this study were fabricated by adhering β-CD to the bare capillaries with PDA. Before coating, the bare capillaries (75 µm i.d., 50.5 cm effective
length and 55.5 cm total length) were conditioned by rinsing sequentially with methanol (10 min), redistilled water (5 min), 0.1 M NaOH (10 min), redistilled water (5 min), 0.5 M HCl (10 min), and finally redistilled water (5 min). Fig. 1 illustrates the preparation process of then coating these conditioned capillaries with β-CD/PDA. Firstly, a certain amount of β-CD was dissolved in 10 mM Tris-HCl buffer solution (pH 8.5); then accurately weighed dopamine was dissolved in the above solution to a final concentration of 5.0 mg/mL. Then, the preconditioned capillary was exposed to the freshly prepared mixture solution of β-CD and DA for 3 min and allowed to stay for 1 h. When dopamine polymerized to form polydopamine (PDA), due to the adhesion properties of PDA, β-CD was immediately fixed on the inner wall of the capillary. Finally, the solution in the separation channel was flushed out and the capillary was rinsed with redistilled water for 5 min to remove residual solution in the channel. The β-CD/PDA coated capillary was filled with redistilled water for storage until use. Throughout chemical modification process, a pressure of 0.07 MPa was applied across the capillary.

A comparable-OT coated capillary column was prepared as follows: (a) the pretreated capillary was exposed to 5.0 mg/mL DA in 10 mM Tris-HCl buffer solution (pH 8.5) for 3 min and allowed to stay for 1 h; (b) 5.0 mg/mL β-CD in 10 mM Tris-HCl buffer solution (pH 8.5) was pumped through the PDA-coated capillary for 3 min and kept for 1 h. The other processes were the same as the above mentioned. The obtained capillary was denoted as β-CD/PDA(2)-coated column.
2.4 Solution preparation

Stock standard solutions of carvedilol and verapamil were extracted with ethanol from medicine tablets to a concentration of 1.0 mg/mL. The other standard solutions of analytes were prepared by dissolving the 1.0 mg amount of each standard analyte in 1.0 mL redistilled water to a concentration of 1.0 mg/mL. All stock solutions were refrigerated at 4°C. Before the CEC analysis, the above stock solutions were diluted to the appropriate concentration with the borate buffer to form the working samples. The working samples were degassed under ultrasonication before injection.

2.5 CEC process

All CEC experiments were performed on a CL1030 system (Beijing Cailu Scientific Instrument, Beijing, China) equipped with an UV (UV-Vis) detector (double light beams, \( \lambda = 190 \sim 720 \) nm, set at 214 nm). Data acquisition and instrument control were carried out on an HW-2000 chromatography workstation (Shanghai Qianpu Software, Shanghai, China). The coated capillary column was rinsed with redistilled water (4 min) and running buffer (5 min) for the first use, and with running buffer (5 min) between the two runs. The sample solution was injected hydrostatically for 3 s at 10 cm height and separated immediately by applying a running voltage of +20 kV. The CEC procedures were performed at room temperature (25°C maintained by air-conditioning). All the solutions including the test samples were filtered through a 0.45 μm pore size cellulose acetate membrane.
At least three runs were performed to obtain one representative electropherogram. And, all RSDs of the migration times of analytes for intra-day, inter-day and column-to-column were calculated based on three consecutive runs (n = 3).

3. Results and discussion

3.1 Characterization of the β-cyclodextrin/polydopamine-coated capillary columns

In the presence of weak base and oxygen, DA can polymerize to PDA by noncovalent self-assembly and by covalent polymerization by its oxidative product, 5,6-dihydroxyindole (DHI) [38]. PDA thus formed readily settles on the inner walls of a capillary column and function as CEC stationary phase[39].

In our work, SEM was firstly used to characterize the morphological structure of the inner wall of β-CD/PDA-coated capillary columns. As we know, the inner wall surface of a bare capillary is very smooth. The SEM images of the inner wall of β-CD/PDA-coated capillary columns with different polymerization time are illustrated in Fig. 2A-C. From these images we can see that the thickness of the inner wall surface increased with increasing polymerization time.

FTIR experiments were carried out to further verify the feasibility of the immobilization of PDA and β-CD on the capillary inner wall by the simple in situ strategy. As can be seen from Fig. 2D, the coated capillary exhibited typical PDA absorption features of aromatic
rings C\(=\)C at 1435, 1500 and 1520 cm\(^{-1}\) and catechol hydroxy at around 3380 cm\(^{-1}\). Besides, C\(=\)O at around 1633 cm\(^{-1}\) indicated that part of the dopamine was oxidized. These bands corresponded to the standard IR spectroscopy of PDA\(^{[40]}\), revealing the formation of PDA film on the capillary inner wall surface. In addition, the absorption bands at 1156 cm\(^{-1}\) and 1080 cm\(^{-1}\) originated from the characteristic absorptions of C–O–C and C–O of β-CD. These results showed that β-CD and PDA were fixed on the capillary inner wall.

Bare capillary and β-CD/PDA-coated capillary columns have different chemical groups on their inner wall surfaces, resulting in differences that can be characterized by streaming potentials (SP)\(^{[41]}\). In this work, the SP measurements were performed on a home-made instrument using the same procedure as Pu et al.\(^{[42]}\). For a bare capillary, the inner wall surface exhibited negative SP over the investigated pH range of 3.0–10.0. However, β-CD/PDA-coated capillary showed modified surface charges compared to bare capillary. As can be seen from Fig. 3, the β-CD/PDA-coated capillary had a positive SP at pH values lower than 6.5, which could be attributed to the covering of Si–OH on the capillary inner surface and the introduction of amino and hydroxyl groups of PDA. Changing in the functional groups of the capillary inner surface can lead to change in the EOF mobility. As shown in Fig. S2 and Table S1, EOF mobility clearly decreased after capillary were coated with PDA and β-CD/PDA. However, for the β-CD/PDA-coated column, the change in EOF mobility was slow, with the polymerization time ranging from 0.5 h to 3.0 h especially for the buffer solutions with pH < 9.0. One possible explanation is that, although the thickness of the
β-CD/PDA coating increased with increasing polymerization time (see Fig. 2A-C), the number of amino and phenolic hydroxyl groups of PDA exposed on the capillary inner wall did not change due to the limited oxygen in the narrow capillary.

The above results of SEM, FTIR, SP and EOF indicated that β-CD and PDA were successfully immobilized on the inner wall of the capillary.

3.2 Mechanism of β-cyclodextrin immobilized on the capillary inner wall

An off-line experiment was designed to verify if there was any polymerization reaction between DA and β-CD, results can be seen in Fig. S3. Clearly, the FTIR spectrum of the off-line product of polymerization between β-CD and DA (A) has the characteristic absorptions of both PDA (B) and β-CD (C); this spectrum is also consistent with the spectrum of the physical mixture of β-CD and PDA (D). In another words, mixing DA and β-CD under such simple conditions did not result in polymerization.

Under the respective optimum conditions of the seven pairs of enantiomers (see Table S2), the β-CD/PDA-coated capillary column showed excellent separation performance (Fig. 4). Separation parameters, including migration time, mobility, selectivity factor, resolution and column efficiency are listed in Table 1. A comparative coating strategy for β-CD/PDA(2) capillary column was designed to further clarify how β-CD was fixed on the inner wall of the capillary column. The performances of the PDA- and β-CD/PDA(2)-coated capillary columns for the chiral analytes were also validated, as shown in Fig. S4. The PDA-coated capillary
clearly had capacity for enantioseparation. While, the β-CD/PDA(2)-coated column readily separated the seven pairs of chiral analytes. These results suggest that β-CD was fixed on the inner surface of the capillary column mainly by the adhesion of PDA. Of course, some other non-covalent interactions may also be occurred, such as hydrogen bonding, π–π stacking and hydrophobic effect.

3.3 Factors affecting the enantioseparation of β-cyclodextrin/polydopamine-coated capillary columns

3.3.1 Effect of CEC separation conditions

It is well known that borate is able to interact with substances containing cis-diol groups such as saccharides and β-CD [43]. This kind of interaction might play a significant role in separation results. To confirm this speculation, borate and phosphate solutions were used as buffer solutions, and their effect on the separation performance of terbutaline enantiomers was examined in the concentration range of 5–15 mM. As seen in Fig. 5A, baseline separation of the terbutaline enantiomers was achieved in 10 mM borate solution. Therefore, 10 mM borate was selected for further study.

Changes in buffer pH has an impact on the ionization degree of analytes and the electrostatic association between the analytes and chiral stationary phase, affecting the separation efficiency. Therefore, we then investigated the effects of buffer pH on the migration time and chiral recognition in the range of pH from 8.00 to 11.00. The
electropherograms under different buffer pH conditions are illustrated in Fig. S5. And their respective optimum buffer pHs of each analyte are displayed in Table S2.

In CEC enantioseparation, organic modifier can influence EOF mobility, change the dissociate constant of analytes, and interfere with the inclusion complexes[44]. In our study, methanol was selected as the organic modifier. As shown in Fig. S6, when the content of methanol increased from 10 to 30% v/v, the separation efficiency of the terbutaline enantiomers was improved gradually and the baseline separation was achieved beyond 20% methanol. However, further increasing methanol content increases migration time due to the reduced EOF mobility (seen in Table S3). Therefore, 20% methanol, representing an optimal trade-off between migration time and enantiomer resolution, was used for our further experiments. For other analytes, their optimum contents of methanol are listed in the Table S2.

To confirm our hypothesis that the change in separation conditions led to the change in the migration order of a pair of enantiomers, the two peaks of tryptophane enantiomers were identified by spiking pure L-tryptophane into the solution of its racemic mixture. As shown in Fig. S7, L-tryptophane interacted more strongly with the immobilized β-CD than D-tryptophane. Furthermore, the migration order of tryptophane enantiomers was not changed with different separation conditions (seen in Fig. S8). Owing to the lack of optically pure standard materials, the elution orders of other enantiomers have not been determined.
3.3.2 Effect of the polymerization reaction conditions

The influence of some polymerization reaction conditions, including DA dosage, β-CD dosage, and the polymerization time on the separation of terbutaline enantiomers was also investigated. Firstly, as can be seen from Fig. S9, when DA dosage was increased from 1.0 to 5.0 mg/mL at the fixed β-CD dosage of 5.0 mg/mL, a more symmetrical separation was achieved. One possible reason could be that 5.0 mg/mL DA facilitated β-CD adherence, which produced more chiral recognition sites on the β-CD/PDA-coated capillary column and result in better enantioseparation. In addition, increasing DA dosage had no effect on EOF mobility (data not shown), which was basically consistent with the results shown in Fig. S2.

Next, when DA was fixed at 5.0 mg/mL, different concentrations of β-CD (including 0.0, 1.0, 2.5, 5.0, 7.5 mg/mL) were used to coat the capillary columns. As shown in Fig. S10, the resolution of the terbutaline enantiomers increased, and a good peak shape was achieved with increasing β-CD concentration in the range of 1.0–5.0 mg/mL. Therefore, the optimum dosage of β-CD was selected at 5.0 mg/mL. Finally, the effect of DA polymerization time on the enantioseparation was also investigated. As illustrated in Fig. S11, good chiral recognition could be obtained for all polymerization times in the range of 0.5–5.0 h. This may be attributed to the quick polymerization process, especially in the narrow capillary column with very small amounts of oxygen. Considering the peak shape and the stability of coated columns, 1.0 h was chosen for the polymerization time.
Besides the chiral recognition capacity of β-CD, the benzene rings, amino and phenolic hydroxyl groups of PDA may favor the enantiomeric separation or selectivity. In other words, the good enantioseparation of β-CD/PDA-coated capillary columns might also be affected by hydrophobic interactions, weak hydrogen bonding and π–π interaction.

3.3.3 Enantioseparation performance of β-cyclodextrin/polydopamine-coated capillary columns

To evaluate the enantioselective ability of β-CD/PDA-coated capillary columns, they were tested with several kinds of enantiomers, including chiral amine drugs, monoamine neurotransmitters and amino acid, as illustrated in Fig. 4 and Table 1. The complete baseline separation ($R_s > 3.4$) of terbutaline enantiomers was achieved. And both verapamil enantiomers and carvedilol enantiomers were separated to a certain extent. The β-CD/PDA-coated capillary column exhibited good separation performance for three monoamine neurotransmitters, namely epinephrine, isoprenaline and norepinephrine (all $R_s$ are close to 1.3). The column also showed chiral recognition capacity for tryptophane enantiomers, as evidenced by good peak shape and resolution ($R_s > 1.5$) in tests. These results showed that the β-CD/PDA-coated capillary column can effectively separated different types of chiral compounds.

The reproducibility of analyte migration time and separation efficiency of β-CD/PDA-coated capillary columns was also investigated. For three consecutive runs, the
RSDs of the migration time of analytes for intra-day, inter-day and column-to-column were in the range of 0.41–1.74%, 1.03–4.18% and 1.66–8.24% (Table S4), respectively. Moreover, the separation efficiency of the β-CD/PDA coated capillary column did not decrease obviously over 90 runs, as seen in Fig. 6. These results indicate that PDA is a perfect adhesion material to introduce and fix β-CD to the inner wall of capillary column.

4. Concluding remarks

We firstly reported a simple, rapid and economical in situ strategy to develop β-CD coated capillary columns at room temperature taking advantage of the adhesion capacity of PDA material. The results of SEM, FTIR, SP and EOF tests indicated that our proposed strategy is feasible for introducing and fixing β-CD on the inner walls of capillaries. The β-CD/PDA coated capillary columns were used for the enantioseparations of the chiral amine drugs, neurotransmitters and amino acid, they performed well, with consistent reproducibility of results. We believe that this work will pave the way to fabricating increasingly useful coated capillary column and will broaden the application of PDA in OT-CEC.

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Electronic Supplementary Information
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Figure captions

Fig. 1. Schematic diagram for the preparation of β-CD/PDA coated capillary column.

Capillary inner wall

Polydopamine

β-CD

R-type

S-type

Tris, pH 8.5, 1 h

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**Fig. 2.** SEM images of the β-CD/PDA coated capillary column with different polymerization time: 1.0 (A), 3.0 (B) and 5.0 h (C). (D) FTIR spectrum of β-CD/PDA modified smashing capillary with polymerization time of 1.0 h.
Fig. 3. The streaming potentials (SP) of bare capillary and β-CD/PDA coated capillary with different polymerization time. Conditions: capillary, 75 μm i.d., 10 cm total length; mobile phase, 2 mM phosphate buffer (pH* 3.0–10.0); the conductivity of all the tested solutions was fixed at 1000 µS/cm.
**Fig. 4.** The electropherograms of the seven pairs of enantiomers. Their respective optimum separation conditions were listed in Table S2. Coating conditions: 5.0 mg/mL DA, 5.0 mg/mL β-CD, 1.0 h of the polymerization time. Injection: hydrostatic injection for 3 s at 10 cm height; Applied voltage: +20 kV; Detection wavelength: 214 nm; Capillary: 75 µm i.d., 55.5 cm total length (50.5 cm effective length).
**Fig. 5.** Effect of the concentrations of borate (A) and phosphate (B) solutions on the separation of terbutaline enantiomers. Separation conditions: pH* 9.5, 20% v/v methanol. Other conditions were the same as in Fig. 4.
Fig. 6. The electropherograms of terbutaline enantiomers with different runs. The experimental conditions were the same as in Fig. 4.
Table 1. The separation parameters for the seven pairs of enantiomers on the β-CD/PDA coated capillary column under their respective optimum conditions.

<table>
<thead>
<tr>
<th></th>
<th>( t_1 )</th>
<th>( t_2 )</th>
<th>( t_0 )</th>
<th>( \mu_0 )</th>
<th>( \mu_{eff1} )</th>
<th>( \mu_{eff2} )</th>
<th>( \alpha )</th>
<th>( R_s )</th>
<th>( N_1 )</th>
<th>( N_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine</td>
<td>8.34</td>
<td>8.46</td>
<td>14.72</td>
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<td>12.1</td>
<td>11.7</td>
<td>1.02</td>
<td>1.45</td>
<td>190336</td>
<td>440250</td>
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<tr>
<td>Norepinephrine</td>
<td>10.34</td>
<td>10.57</td>
<td>14.72</td>
<td>1.59</td>
<td>6.7</td>
<td>6.2</td>
<td>1.06</td>
<td>1.59</td>
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<tr>
<td>Isoprenaline</td>
<td>10.68</td>
<td>10.91</td>
<td>14.72</td>
<td>1.59</td>
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<tr>
<td>Terbutaline</td>
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<td>2.08</td>
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<td>4.2</td>
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<td>2.22</td>
<td>53833</td>
<td>64718</td>
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<tr>
<td>Verapamil</td>
<td>10.53</td>
<td>10.80</td>
<td>16.95</td>
<td>1.38</td>
<td>8.4</td>
<td>7.8</td>
<td>1.04</td>
<td>1.41</td>
<td>61451</td>
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<tr>
<td>Carvedilol</td>
<td>9.99</td>
<td>10.15</td>
<td>13.86</td>
<td>1.68</td>
<td>6.5</td>
<td>6.2</td>
<td>1.04</td>
<td>1.23</td>
<td>153581</td>
<td>101465</td>
</tr>
</tbody>
</table>

\( t \): the migration time (min);

\( \mu_0 \): the mobility of the electroosmotic flow (cm\(^2\) V\(^{-1}\) s\(^{-1}\)), \( \mu_0 = \frac{IL}{Vt_0} \), where \( I \) and \( L \) are the effective and total lengths of the capillary; \( V \) is the applied voltage.

\( \mu_{eff} \): the effective mobility of the enantiomer (cm\(^2\) V\(^{-1}\) s\(^{-1}\)), \( \mu_{eff} = \frac{IL}{V} \left( \frac{1}{t_2} - \frac{1}{t_1} \right) \).

\( \alpha \): the selectivity factor, \( \alpha = \frac{\mu_{eff1}}{\mu_{eff2}} \).

\( R_s \): the resolution, \( R_s = 2 \times \left( t_2 - t_1 \right) / \left( W_1 + W_2 \right) \), where \( W \) is the peak width (min).

\( N \): the theoretical plate number, \( N = 5.54 \times \left( \frac{t}{W_{1/2}} \right)^2 \), where \( W_{1/2} \) is the peak width at half height (min).

0, 1 and 2 are denoted as the EOF marker, the first and second peak of the enantiomers, respectively.