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Full Paper

Sensitive Method for Enantioseparation of Rivastigmine with Highly Sulfated Cyclodextrin as Chiral Selector by Capillary Electrophoresis

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A sensitive method for enantioseparation of a basic drug rivastigmine and determination of its optical impurity by capillary electrophoresis with highly sulfated *β*-cyclodextrin (HS-*β*-CD) as the chiral selector is described. In general, enantioseparation of basic chiral compounds is carried out in acidic condition (pH 2.5) to prevent analytes from adsorption on the capillary wall. However, in the case of rivastigmine, the detection sensitivity was too limited to determine the optical impurity of *S*-rivastigmine lower than 1% when buffer pH was 2.5. It was found that the detection sensitivity was improved 1.6 times just by raising the buffer pH value from 2.5 to 5.8. The poor column efficiency due to the adsorption of the analytes on the capillary wall was resolved by using dynamical coating of the capillary wall with the linear polyacrylamide solution. The experimental parameters such as the concentration of HS-*β*-CD, buffer pH and buffer ionic strength were optimized, respectively. The method was validated in terms of repeatability, linearity, limit of detection (LOD) and limit of quantitation (LOQ). Using the present method, the optical purity of nonracemic rivastigmine with the enantiomeric excess (ee) value of 99.14% was determined.

Keywords rivastigmine, enantioseparation, capillary electrophoresis, highly sulfated *β*-cyclodextrin

Introduction

It is well recognized that the enantiomers of chiral drugs may display different pharmacological activities. One enantiomer is therapeutically effective while the other may be less effective or even toxic. Currently, at least 60% of the drugs in the market possess chirality, and about 48% of the chiral drugs were sold as the single enantiomer.[1] To ensure the safety of the chiral drugs, it becomes more and more urgent to develop effective enantioseparation methods for assaying the optical impurity of chiral drugs for quality control and clinic investigation.

Capillary electrophoresis (CE) has been accepted as a powerful separation tool for enantioseparation.[2-4] Compared with high performance liquid chromatography (HPLC), CE has several advantages such as shorter analysis time, higher separation efficiency, simple methodology and lower operational cost. In CE, the enantioseparation is achieved by using chiral additives, which is dissolved in the running buffer. During the last ten several years, various types of chiral selectors have been successfully used in CE, such as cyclodextrins (CDs) and their various derivatives,[5] glycopeptide antibiotics including vancomycin and teicoplanin,[6-8] chiral micelles,[9] metal chelate complexes,[10] chiral crown ether[11] and *etc*. Among these chiral selectors, CDs and their derivatives represent the most commonly used chiral selectors for enantioseparation with CE.[12] It has been established that the charged CDs, especially the highly sulfated CDs, can display superior enantioselectivity to neutral CDs in most cases.[2,13-16] It may be due to the reason that the charged CDs can offer not only the inclusion complexation interaction, but also the strong electrostatic interactions. Furthermore, the neutral chiral compounds can be resolved by CE using the charged CDs.

Rivastigmine, (*S*)-3-[1-(dimethylamino)ethyl]phenyl ethyl(methyl)carbamate hydrochloride (Figure 1), a carbamate type inhibitor of acetylcholinesterase, is therapeutically used for the treatment of Alzheimer's disease.[17] It proved that the *S*-enantiomer is more pharmacologically potent than the *R*-form.

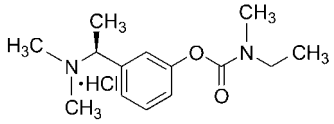

Figure 1 Chemical structure of *S*-rivastigmine hydrochloride.

Several achiral gas chromatography and liquid chromatography methods have been reported for simultaneous determination of rivastigmine and its major

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metabolites.[18-21] Recently, Kavalírová[22] and Kenndler[23] separated the racemic rivastigmine by CE with CD modified buffer at pH 2.5. However, when we tried to determine the optical impurity of *S*-rivastigmine with the reported methods, we found that the detection sensitivity was not sufficient to assay the minor enantiomer *R*-rivastigmine lower than 1% at pH 2.5.

The aim of this work is to develop a method with improved detection sensitivity and separation efficiency for assaying the optical purity of *S*-rivastigmine samples. In order to improve the detection sensitivity, running buffer with pH 5.8 was used, because we found that the detection sensitivity was improved 1.6 times by raising the running buffer pH from 2.5 to 5.8. Dynamic coating technique with linear polyacrylamide solution was used to improve the separation efficiency caused by the adsorption of analyte on the capillary wall. Moreover, method validation in terms of repeatability, linearity, LOD and LOQ was carried out.

Materials and methods

Instrumentation

All CE separations were performed with a Beckman P/ACE MDQ capillary electrophoresis system (Fullerton, CA, USA) equipped with a UV detector. Fused silica capillary with a dimension of 50 μm I.D. (370 μm O.D.)×39 cm (29 cm to detection window) was purchased from Yongnian Optical Fiber Inc. (Hebei, China). Column was thermostatted at 15 ℃. Applied voltage was 15 kV. Samples were injected by pressure at 1379 Pa for 5 s, and were detected at 200 nm. Ultraviolet absorption spectrum of rivastigmine dissolved in buffer was measured using Ultraviolet and Visible spectrophotometer (Varian Cary 100, USA).

Reagents

All rivastigmine samples were from Shanghai Sunve Pharmaceutical Co., Ltd. (Shanghai, China). HS-*β*-CD (with degree of substitution *ca*. 7—11) was obtained from Aldrich (Steinheim, Germany), and *N*,*N*,*N'*,*N'*-tetramethylethylenediamine (TEMED) was obtained from Fluka (Buchs, Switzerland). Tris(hydroxymethyl)-aminomethane (Tris), acrylamide, ammonium persulfate (APS) were purchased from Shanghai Shisheng Biotechnological Inc. (Shanghai, China). All reagents were of analytical grade. The buffer pH was adjusted to the desired value with the phosphoric acid solution prior to addition of CD. The buffer was filtered through a 0.45 μm nylon filter prior to use. The stock solutions for (±)-rivastigmine and *S*-enantiomer with the concentration of 2 mg/mL were prepared with deionic water, respectively.

Procedure

A linear polyacrylamide (LPA) solution 4 mg/mL was prepared according to a protocol reported in the literature.[25] Briefly, 0.20 g of acrylamide was dissolved in 5 mL of 50 mmol•L^{-1} Tris-phosphate buffer at pH 5.8, and degassed with ultrasound for 15 min. The polymerization was initiated by addition of 50 μL of 10 mg/mL APS and 20 μL of 10%(*v*/*v*) TEMED. The polymerization was allowed to complete over 24 h at 4 ℃, without stirring. Prior to use, the polymer solution was diluted by 10 times with the running buffer to get a less viscous and homogeneous solution. Dynamic coating was performed by the following procedure: washing the capillary with 0.1 mol•L^{-1} NaOH, 0.1 mol•L^{-1} HCl and water for 3 min, respectively, followed by rinsing the capillary with the diluted LPA for 5 min and leaving the polymer solution inside the capillary for another 5 min. Finally, the capillary was rinsed with the running buffer for 2 min with lower pressure. Between two runs, the capillary was dynamically coated with the LPA solution.

Results and discussion

Method development and optimization

Tris-phosphate buffer at pH 2.5 is the favorite buffer for enantioseparation of basic chiral compounds with CE, because the adsorption of the analytes on the capillary wall can be diminished significantly, and the low electroosmaticflow (EOF) is favorable to gaining the high resolution. Although the enantioseparation of racemic rivastigmine with CE has been reported in the literature,[22,23] however, we found that their detection sensitivity was not high enough to be able to determine the minor R-enantiomer lower than 1%. As shown in Figure 2, the UV absorption of rivastigmine obtained at pH 5.8 displayed 1.6 times higher than that of pH 2.5 at 200 nm. Therefore, pH 5.8 was used in our experiment to improve the detection sensitivity. However, the poor separation efficiency was observed when pH 5.8 was used due to the adsorption of the analyte on the capillary wall. In order to suppress the adsorption, dynamically coated the capillary with LPA was utilized. As predicted, the coated capillary provided a stable EOF between pH 5.5—7.0 as shown in Figure 3, and the column efficiency and resolution factor were improved 1.7 and 1.4 times, respectively. In the whole investigated pH range from 5.5 to 6.5, rivastigmine could be baseline separated with resolution factors higher than 2.8. pH 5.8 was selected as the separation condition because of the best resolution factor.

The effect of Tris concentration on the separation of rivastigmine was investigated in a range from 30 to 70 mM. Racemic rivastigmine could be baseline separated in the whole studied buffer concentration. 50 mM was chosen as the optimal condition for the following experiment because of the best resolution factor.

The influence of the HS-*β*-CD concentration on the separation was investigated in a range from 0 to 24 mg/mL. As shown in Figure 4, the resolution factors were increased with the increase of the HS-*β*-CD concentration. The resolution factor of 2.2 was achieved at

the concentration of 18 mg/mL. Therefore, 18 mg/mL was selected as the optimal separation condition.

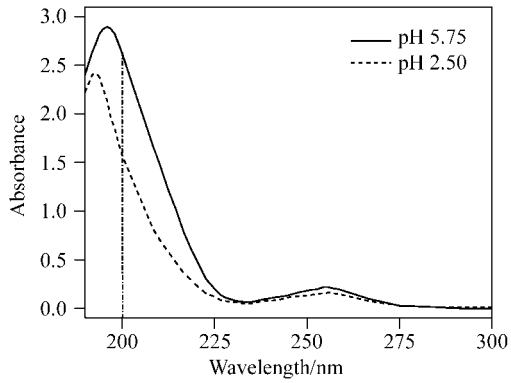

Figure 2 Comparison of the UV absorption spectra of rivastigmine obtained under different pH conditions with racemic rivastigmine concentration of 0.2 mg/mL in 60 mmol•L^{-1} Tris buffer containing HS-β-CD 18 mg/mL.

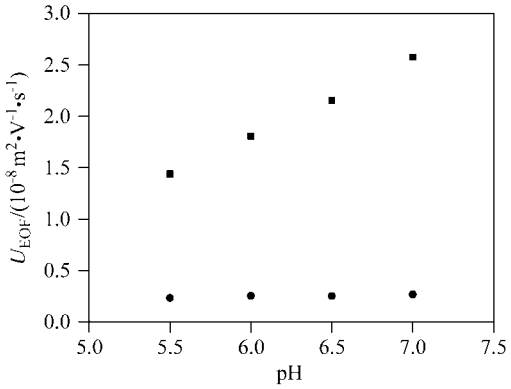

Figure 3 EOF mobility variation versus pH for untreated (■) and LPA dynamically coated (●) capillary.

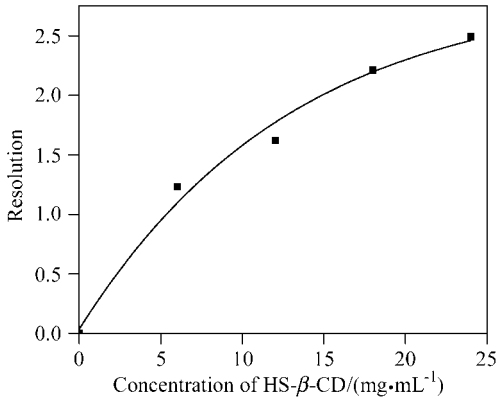

Figure 4 Effect of concentration of HS-β-CD on enantioseparation. Conditions: background electrolyte, 50 mmol•L^{-1} Tris buffer (pH 5.8) containing various amount of HS-β-CD, applied voltage was 15 kV, column temperature was 15 ℃.

In our experiments, the stability of LPA coating was also studied. It was proved that the coating function was stable at least for 22 times of separation. The repeatability in terms of migration time and resolution factors was 1.1% and 4.8% over 22 times separation, respectively.

Finally, the overall optimal conditions were established as 50 mmol•L^{-1} Tris buffer (pH 5.8) containing 18 mg/mL HS-β-CD at 15 kV applied voltage. The electropherograms of the enantioseparation of a racemic and a non-racemic rivastigmine samples are shown in Figure 5.

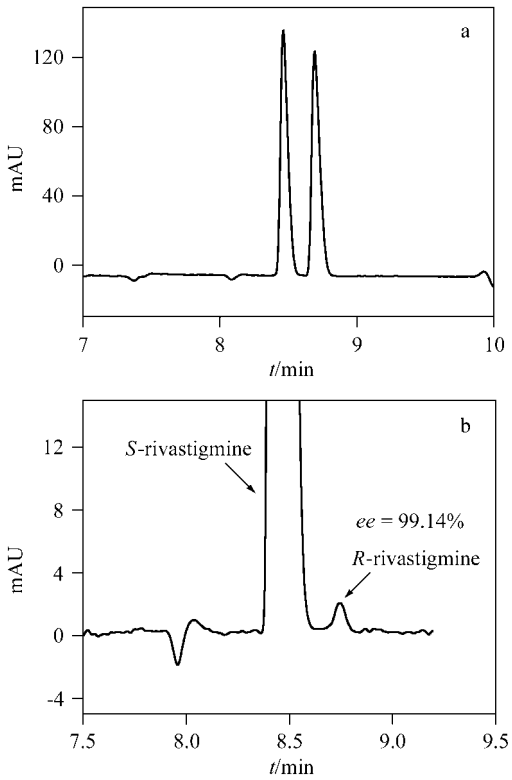

Figure 5 Electropherograms of enantioseparation of racemic (a) and non-racemic (b) rivastigmine samples. Conditions: 50 mmol•L^{-1} Tris buffer (pH 5.8) containing 18 mg/mL HS-β-CD, concentration of samples was 0.4 mg/mL each. Sample was injected by pressure at 1379 Pa for 5 s.

Method validation

In order to determine the optical impurity of *S*-rivastigmine samples, the method must be validated in terms of linearity, LOD and LOQ repeatability,. The results are summarized in Table 1. Single enantiomer *S*-rivastigmine was used to establish the linearity over the concentration range from 0.003 to 1.5 mg/mL (6 points). At the top concentration of the calibration curve, the racemic rivastigmine sample can be still baseline separated attributed to the high selectivity of the present method. Further, an *S*-rivastigmine sample of 0.2 mg/mL was used for intraday and interday repeatability study. The LOQ and the LOD corresponding to signal-to-noise of 10 and 3, respectively, were determined with the racemic sample. For both enantiomers, the LOQ and LOD were determined as 0.0014 and 0.0004 mg/mL, respectively. In the present method, the LOQ is approximately 1.6 times lower than that of the literatures.[22,23] Finally, the method was applied to determine

the optical purity of a nonracemic rivastigmine sample with the ee value of 99.14% (shown in Figure 5).

Table 1 Validation data for *S*-rivastigmine[a]

Intraday repeatability (n=6)	
Migration time	RSD=0.40%
Peak area	RSD=0.37%
Interday repeatability (n=3)	
Migration time	RSD=1.1%
Peak area	RSD=2.9%
Linearity	y=27264 x+399.9 (R^2=0.9989)
LOQ (S/N=10)	0.0014 mg/mL
LOD (S/N=3)	0.0004 mg/mL

[a] n is numbers of determinations; S/N is the ratio of sign to noise, Injection volume was calculated as 3 nL; y is peak area, x isconcentration in mg/mL, number of concentration points is 6, each point was measured in triplicate. Conditions: background electrolyte, 60 mmol•L^{-1} Tris buffer (pH=5.8) with 18 mg/mL HS-β-CD.

Stability of rivastigmine in solution was studied by keeping the solution in volumetric flask at room temperature for 3 d and measuring the impurity with a 24 h interval. The result displayed that neither extra peak, nor racemization happened.

Conclusion

A method for determining the optical impurity of *S*-rivastigmine with improved detection sensitivity and separation efficiency has been developed. It was found that the detection sensitivity was improved by raising the running buffer pH. Dynamically coating the capillary with LPA was successfully used to improve the separation efficiency and the reproducibility. The optical impurity of a real sample was determined with the present method successfully.

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