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Enantiomeric separation of naphthalene-2,3-dicarboxaldehyde derivatized DL-3,4-dihydroxyphenylalanine and optical purity analysis of L-3,4-dihydroxyphenylalanine drug by cyclodextrin-modified micellar electrokinetic chromatography

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Abstract

A new cyclodextrin-modified micellar electrokinetic chromatography (CD-MEKC) method for the enantiomeric separation of 3,4-dihydroxyphenylalanine (DOPA), derivatized with naphthalene-2,3-dicarboxaldehyde (NDA) to produce cyanobenzoisoindole (CBI) derivative, has been developed. The unsubstituted α-, β-, γ-cyclodextrins (CDs) and hydroxypropyl-substituted α-, β-, γ-CDs, as chiral selector, were examined for the enantiomeric separation of CBI-DL-DOPA. In addition to the concentration of chiral selector, some other experimental factors also have been optimized, such as concentration of borate buffer, content of sodium dodecyl sulphate (SDS), pH of electrolyte and applied voltage. Optimal separation was obtained at pH 8.0, 100 mM borate solution containing 5 mM HP-γ-CD and 120 mM SDS, as well as 18 kV applied voltage and 25 °C capillary temperature. Detection was followed by direct UV absorptiometric measurements at 254 nm. The developed method was employed for optical purity analysis of levodopa drug and allowed the determination of 0.14% D-DOPA in L-3,4-dihydroxyphenylalanine (levodopa) with well peak identification.

Keywords: Micellar electrokinetic chromatography; Enantiomeric separation; 3,4-Dihydroxyphenylalanine; Cyclodextrin

1. Introduction

L-3,4-Dihydroxyphenylalanine (L-DOPA) has been considered to be an inert amino acid that alleviates the symptoms of Parkinson's disease by its conversion to dopamine via the enzyme aroma L-amino acid decarboxylase. In contrast to this generally accepted idea, Yoshimi et al. [1] proposed that DOPA itself is a neurotransmitter and/or neuromodulator in addition to being a precursor of dopamine at recent. However, D-DOPA has toxic properties [2], and use of racemic mixtures containing L-DOPA and D-DOPA may cause serious side effects because of the different metabolisms of the active and inactive components [3]. Therefore, it is having an increase in the demand for enantiomeric separation and the determination of enantiomeric impurity in connection with the synthesis of L-DOPA and with biological, pharmacokinetic and clinical investigations of L-DOPA.

In the past decade, many efforts have been focused on the separation and the determination of L-DOPA by high-performance liquid chromatography (HPLC) [4–6] and capillary electrophoresis (CE) [7–9] in various biological samples. However, only a few reports are involved in enantiomeric separation of DL-DOPA and the determination of D-DOPA. Lou et al. [10] reported a method for direct enantiomeric separation of DL-DOPA and their intermediates by supercritical fluid chromatography. Milada and Magda [11] reported the determination of the enantiomeric purity of L-3,4-dihydroxyphenylalanine (levodopa) by HPLC. Although

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DOPA enantiomers have also been successfully separated by CE using chiral crown ethers [12] and charged cyclodextrins (CDs) such as sulfobutyl ether-β-CD [13], heptakis (2,3-diacetyl-6-sulfato)-β-CD [14] and sulfated-β-CD [15], but as far as we know, only one CE procedure has been reportedly used for the determination of enantiomeric purity in pharmaceuticals [16].

Guang and Mario [17] derivatized DL-DOPA with Marfey's reagent and separated the derivatized compounds by means of micellar electrokinetic capillary chromatography (MEKC). MEKC, which was first proposed by Terabe et al. [18], is even more promising as it can be used for selective separation of both neutral and ionic compounds [19]. This technique involves the addition of surfactants to the operating buffer in a higher amount than the critical micelle concentration. The solute is then partitioned between the micelle and the surrounding aqueous medium. Cyclodextrin-modified micellar electrokinetic chromatography (CD-MEKC), a variation of MEKC, is developed by Terabe and co-workers [20,21]. The use of aqueous solutions containing micelles and CDs as moving phases in CE not only can separate neutral and charged compounds, but also enables the enantioseparation of chiral compounds. This approach has been widely reported for the enantioseparation of amino acid derivatives [22–25]. Derivatization of amino acids is of general use to enable UV–vis or fluorescence detection and to enhance chiral discrimination mechanisms [26]. Naphthalene-2,3-dicarboxaldehyde (NDA) is one of the most widely used derivatization reagent for amino acids. This reagent reacts rapidly with amino acids in the presence of cyanide to form cyanobenzoisoindole (CBI) derivatives. The corresponding CBI-derivatives are sufficiently stable for enantiomeric separation. And a series of reports on enantioseparation of CBI derivatives by CD-MEKC have been appeared [27–30].

In this work, NDA was used to derivatize the DOPA, which enhances greatly the separation efficiency and detection sensitivity of DL-DOPA. And a new CD-MEKC method for the enantiomeric separation of DL-DOPA has been developed. Several unsubstituted α-, β-, γ-cyclodextrins (CDs) and hydroxypropyl substituted α-, β-, γ-CDs (HP-α-CD, HP-β-CD, HP-γ-CD), as chiral selector, were investigated. The enantiomeric separation of CBI-DL-DOPA can be achieved by using HP-γ-CD as chiral selector in a running time less than 20 min. Determination of enantiomer impurity of levodopa drug by using the present method is well shown.

2. Experimental

2.1. Reagents

NDA was purchased from Molecular Probes (Eugene, OR, USA). DL-DOPA, D-DOPA and L-DOPA were purchased from Fluka (Switzerland). α-, β-, and γ-CDs were purchased from Sigma (St. Louis, MO, USA). HP-CDs (α-, β-, and γ-) were purchased from Aldrich (Milwaukee, WI, USA). Sodium dodecyl sulphate (SDS) was purchased from Shanghai Chemical Reagent (Shanghai, China). Levodopa drugs were from Hefeng pharmaceutical company (Guangxi, China). All other chemicals and organic solvents used in this work were of analytical grade. Analytes were dissolved in 0.1 M HCl solutions. Doubly distilled water was used throughout.

2.2. Capillary electrophoresis

A HP^{3D} capillary electrophoresis system (Hewlett Packard, Waldbronn, Germany) equipped with a photodiode array detector (190 nm–600 nm) was used in this CE separation. Uncoated fused-silica capillaries 55 cm (effective length 46 cm) × 50 μm were used for separation. Detection was a fixed wavelength of 254 nm with date processed on a HP chemstation. The pH values of running buffer were measured with a Model pHs-3C pH meter (Leici factory, Shanghai, China). New uncoated capillaries were conditioned by flushing with 1 M and 0.1 M NaOH for 30 min each, followed by 10 min of doubly distilled water before use. Between two consecutive injections, the capillary was rinsed sequentially doubly distilled water and running buffer for 3 min each. Samples were injected into capillary by applying hydrodynamic pressure (50 mbar) for 10 s. The running buffer solution was prepared by dissolving the appropriate amounts of HP-γ-CD and SDS in a 100 mM borate buffer solution (pH 8.0). All solutions were filtered through a 0.45-μm pore-size membrane filter before use. The separation voltage used was 18 kV. Capillary temperature was 25 °C.

2.3. Precolumn derivatization

DOPA standard solution (10 μL) was transferred to a 0.5 mL derivatization vial, and 0.1 M borate buffer solution (pH 9.0, 150 μL) was added. Derivatizing reagents, NDA (20 mM in methanol, 50 μL) and KCN (20 mM in water, 50 μL) were added to the vial in sequence, and the vial was capped. After gentle shaking, the solution was allowed to keep at room temperature for 30 min. The derivative solution was injected for separation without additional purification.

2.4. Determination of enantiomer impurity in levodopa drug

One tablet of levodopa drug (containing 250 mg of L-DOPA) was smashed and dissolved in 10 mL of 0.1 M HCl solution. The solution was centrifuged (2000 rpm) for 10 min, and the centrifuged solution was diluted to 250 mL with 0.1 M HCl solution. Ten microliters of the solution was derivatized as described above. The derivative solution was used for separation and determination.

3. Results and discussion

3.1. Choice of chiral selector

Enantioseparation of CBI-DL-DOPA in CD-MEKC is obtained through formation of an inclusion complex with CD, and SDS monomers can have their hydrophobic tails co-included in the CD cavity along with the CBI-DOPA [31]. Therefore, the size of the nonpolar cavity of CD is a critical factor for formation of the inclusion complex. In this study, α-CD, β-CD, γ-CD, HP-α-CD, HP-β-CD and HP-γ-CD were chosen to determine which CD forms inclusion complex most effectively with CBI-DOPA and SDS monomers. As Fig. 1 shows, the migration time of CBI-DOPA was about 13 min in the absence of CD under the experimental conditions (Fig. 1a). When 5 mMα-CD or HP-α-CD was added into running buffer, no change in the migration time was observed, which indicates that α-CD and HP-α-CD do not interact significantly with CBI-DOPA. When 5 mM β-CD or HP-β-CD was added, the migration time was increased to about 22 min, which suggests that CBI-DOPA forms an inclusion complex with these CDs, however, little chiral separation was observed (Fig. 1c). CBI-DL-DOPA was enantioseparated by using a running buffer containing γ-CD or HP-γ-CD, but the baseline enantioseparation for CBI-DL-DOPA was observed only by using HP-γ-CD as chiral selector (Fig. 1b). It indicated that the size of the cavity of γ-CD should be more suitable for CBI-DOPA to form effective inclusion complex than other kinds of CD in the presence of SDS monomers.

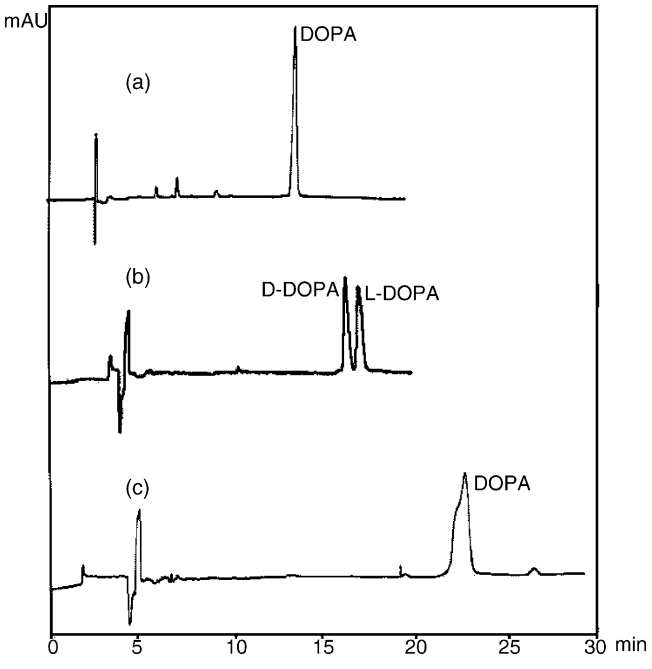

Fig. 1. Chromatogram of CBI-DL-DOPA. Electrolyte composition was 100 mM borate buffer (pH 8.0) containing 120 mM SDS (a); 120 mM SDS and 5 mM HP-γ-CD (b); 120 mM SDS and 5 mM β-CD (c). Capillary 55 cm × 50 μm i.d.; applied voltage 18 kV; capillary temperature 25 °C; UV detection wavelength 254 nm.

Furthermore, the hydrogen bonding ability of HP-γ-CD also plays an important role.

3.2. Effect of pH of running buffer

In CE chiral separation, changes in running buffer pH may cause changes in the charges of analytes and/or chiral selectors. This will lead to changes chiral separation selectivity. In this separation system, pH affects directly the ionization of CBI-DOPA. In weak basic running buffer, CBI-DOPA remains negatively charged, which increases its interaction time with HP-γ-CD. Therefore, a borate buffer in the pH range of 7.5–9.5 was used to study the effect of pH. As a result, the resolutions were more than 2.0 under all investigated pH values except for pH 7.5 with a resolution of 1.98, and the resolution reaches a maximum in the pH range of 7.8–8.2. With increase of pH value in running buffer below pH 7.8, it was found the increase of the migration time of CBI-DOPA. This is due to the CBI-DOPA being ionized (negatively charged) as pH increases; negatively charged CBI-DOPA migrates in the opposite direction to the EOF. But, after pH value of running buffer was higher than 8.2, EOF is maximized and results in a short migration time of CBI-DOPA, which again cause the resolution decrease slightly (Fig. 2).

3.3. Effect of running buffer concentration

After optimizing the pH of the running buffer, another series of experiments were performed at the optimal pH of 8.0 with 5 mM HP-γ-CD and 120 mM SDS to determine the optimum running buffer concentration. Five borate buffer concentrations, namely 30, 50, 80, 100 and 120 mM were chosen to investigate the effect of running buffer concentration on the resolution and migration time. As can be seen from Table 1, the migration time of CBI-DOPA increases with the running buffer concentration increasing, and the resolution also increases gradually. But the resolution decreases slightly after

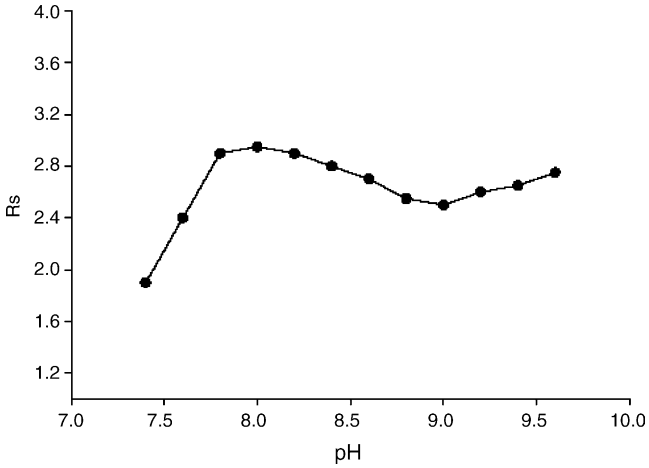

Fig. 2. Effect of running buffer pH on the resolution of CBI-DL-DOPA. CE conditions: 100 mM borate buffer (pH 8.0) containing 120 mM SDS and 5 mM HP-γ-CD. Other conditions as in Fig. 1.

Table 1
Dependence of migration time and resolution on borate concentration and applied voltage

	D-Enantiomer migration time (min)	Resolution
Applied voltage 18 kV, borate concentration (mM)		
30	10.42	1.51
50	11.96	1.89
80	13.85	2.36
100	16.30	2.87
120	18.87	2.75
Applied voltage (kV); borate concentration 100 mM		
15	22.81	2.80
18	16.30	2.87
20	14.85	2.57
22.5	12.59	2.12

Other separation conditions: pH 8.0; 5 mM HP-γ-CD; 120 mM SDS; capillary temperature 25 °C. UV detection wavelength 254 nm.

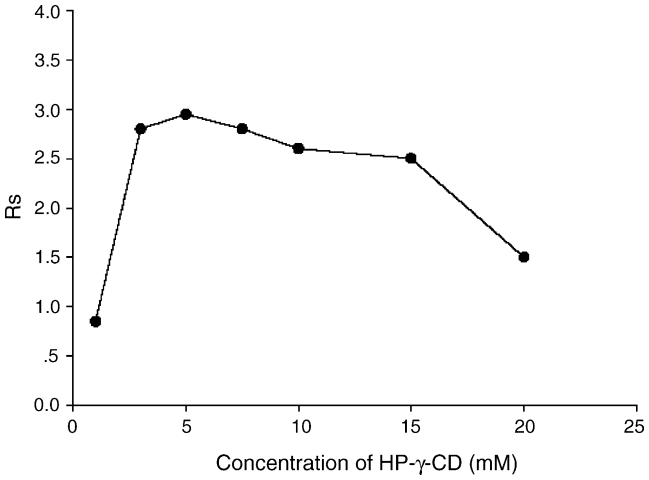

Fig. 3. Effect of HP-γ-CD concentration on the resolution of CBI-DL-DOPA. CE conditions: 100 mM borate buffer (pH 8.0) containing 120 mM SDS and 1–20 mM HP-γ-CD. Other conditions as in Fig. 1.

the concentrations were higher than 100 mM. Furthermore, the eluted peak shape of CBI-DOPA became worsened after the concentrations were higher than 120 mM due to produce large Joule heating. Thus, 100 mM borate buffer is considered optimal.

3.4. Effect of HP-γ-CD concentration

The effect of HP-γ-CD concentration on the chiral separation of CBI-DL-DOPA was investigated over the concentration range 1–20 mM. As illustrated in Fig. 3, the resolution of CBI-DL-DOPA increases dramatically with increasing HP-γ-CD concentration from 1 mM to 3 mM, and forms a platform at a range of 3 mM to 5 mM where maximum resolution was reached. Further, increasing HP-γ-CD concentration results in a decrease of resolution, which is due to the migration time decreases with increasing HP-γ-CD concentration from 5 mM to 20 mM. Therefore, we can estimate the optimum HP-γ-CD concentration for enantioseparation of CBI-DL-DOPA to be approximately 5 mM.

3.5. Effect of SDS concentration

Owing to the NDA derivatives having strong hydrophobicity, therefore having SDS present in running buffer, reveals the importance for the enantioseparation of CBI-DL-DOPA. On the other hand, SDS monomers can have their hydrophobic tails co-included in the CD cavity along with the CBI-DOPA. This could change the nature of CBI-DOPA/CD interaction (its binding constant and stereoselective fit) and the resolution. In the absence of SDS, the resolution of CBI-DL-DOPA is very poor ($R_s = 0.55$) under the experiment conditions. After SDS was added to the running buffer, the continuous increase of the separation selectivity was observed with increasing SDS concentration. However, higher peak dispersion and electric current together with impracticable long analysis time at higher SDS concentrations were the reasons why we selected 120 mM SDS as the optimal one.

3.6. Effect of applied voltage

Since higher resolution should be achieved when more time is available for diffusion-controlled interaction between the analyte and the chiral selector, use of lower voltage was expected to improve resolution. Dependence of resolution on applied voltage is summarized in Table 1. Obviously, it would be ideal to a low applied voltage of 18 kV as a compromise to obtain shorter migration time and to reach higher resolution.

3.7. Determination of enantiomeric impurity in levodopa drug

In order to examine whether the proposed method could be acceptable for the optical purity analysis of levodopa, the method was checked for the repeatability of migration times and peak areas, linearity, limits of detection, precision and accuracy.

Table 2
Repeatability of the migration times and the peak area percentage of racemic dopa

	First enantiomer (R.S.D. %; $n = 5$)		Second enantiomer (R.S.D. %; $n = 5$)	
Average migration time (min)	16.30	(2.8)	16.92	(1.7)
Average peak area (%)	50.6	(1.2)	49.4	(1.0)

Separation conditions: 100 mM borate (pH 8.0) containing 5 mM HP-γ-CD, 120 mM SDS; applied voltage 18 kV; capillary temperature 25 °C. UV detection wavelength 254 nm.

Table 3
Precision and accuracy results of the proposed method

D-DOPA concentration (%)	Mean calculated concentration (%)	Precision (R.S.D. %, $n = 6$)	Accuracy (%) (deviation of means from calibration curve)
0.1	0.096	2.6	95.7
0.5	0.48	1.0	98.4
1.0	1.02	1.6	103
1.5	1.49	1.4	99.6
2.0	1.98	2.3	98.2

Separation conditions as in Table 2.

The repeatability of migration times and peak areas of the enantiomers of CBI-DL-DOPA were investigated by injecting CBI-DL-DOPA solution five times under optimum separation conditions. The results obtained in the study are summarized in Table 2. As can be seen, a good repeatability on migration times and peak areas of CBI-DL-DOPA can be obtained by this method.

L-DOPA was derivatized and separated alone, and no trace of D-DOPA was detectable. The racemic DOPA was added into the standard L-DOPA in the range of 0.10%–2.0% to investigate the linearity. Linear regression analysis of the results yielded the following equation: $Y = 0.6601X + 0.0064$ ($r = 0.9992$), where Y is the peak area of CBI-D-DOPA, X is the percentage of the enantiomeric impurity, and r is the correlation coefficient.

The limit of detection (LOD) of the method, corresponding to signal-to-noise ratios of 3, was calculated from linear regression analysis made by plotting the analyte peak height versus the percentage of the enantiomeric impurity. The LOD, the lowest concentration of D-DOPA that could be detected, was 0.04%, which corresponds to concentration of CBI-D-DOPA of 0.15 μg/mL (about 8×10^{-7} M).

The precision and accuracy of the method were investigated by injecting CBI-L-DOPA solutions containing 0.10%–2.0% D-DOPA six times; the results were summarized in Table 3. These validations showed that the proposed method is suitable for the determination of the enantiomeric impurity in L-DOPA.

A commercial sample of levodopa drug was analyzed for enantiomeric impurity. Content of D-DOPA in levodopa was calculated with reference to the calibration curve described previously. The result obtained by three parallel determination showed containing 0.14% D-DOPA in levodopa. A typical chromatogram obtained from a commercial sample of levodopa drug was shown in Fig. 4a. The two peaks corresponding to D-DOPA and L-DOPA can well identify. To verify the peak identification, 0.5% D-DOPA was added into levodopa sample, and then the sample was again derivatized and separated. As can be seen by comparing Fig. 4a with Fig. 4b, the D-DOPA peak increases in size without other major changes in the chromatogram, and according to the increased peak area a recovery of 95% was obtained. It is worth noting that the D-DOPA level in levodopa as measured by the present method is similar with the results reported in Ref. [32], which was obtained by an HPLC procedure.

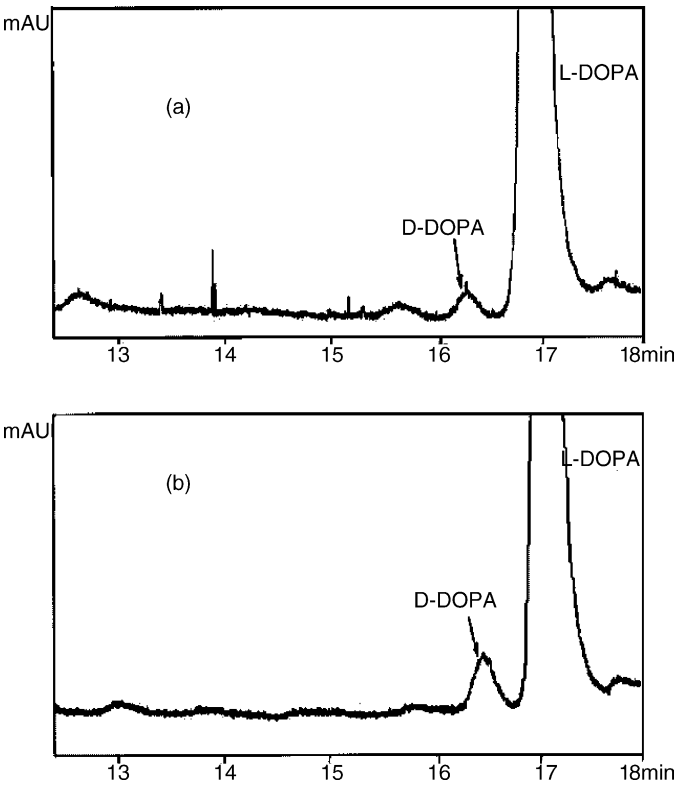

Fig. 4. Chromatogram of commercial sample of levodopa drug: the levodopa tablets sample (a); the levodopa tablets sample was spiked with 0.5% D-DOPA (b). CE conditions as in Fig. 1b.

4. Conclusion

A new CD-MEKC method for the enantiomeric separation of DL-DOPA has been developed. In this method, NDA was used to derivatize DOPA, which enhances greatly the separation efficiency and detection sensitivity of DL-DOPA. HP-γ-CD was selected as the most effective chiral selector, and a high resolution of CBI-DL-DOPA enantiomers with electrophoresis time of around 17 min was achieved. The method has the advantage of being simple, reproducible and more sensitive than many reported methods. It is well suited

for the determination of enantiomer impurity in levodopa drug.

Acknowledgements

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