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ORIGINAL PAPER

Determination of Enantiomeric Impurity of Levamlodipine Besylate Bulk Drug by Capillary Electrophoresis Using Carboxymethyl-β-Cyclodextrin

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Abstract A rapid capillary electrophoresis method using carboxymethyl-β-cyclodextrin (CM-β-CD) as chiral selector was developed and validated for the enantiomeric purity determination of levamlodipine besylate bulk drug. Several parameters for were optimized for a satisfactory enantioresolution, including pH of background electrolyte, the concentration of chiral selector, buffer concentration, capillary temperature and voltage. The highest resolution (Rs = 9.8) was obtained with 4 mM CM-β-CD dissolved in 40 mM phosphate buffer (pH 3.5), at temperature 25 °C and voltage 30 kV, normal polarity. This method was fully validated for the enantiomeric purity determination of the R-amlodipine at the 0.2 % level. The established method was validated in terms of selectivity, LOD and LOQ (0.001 and 0.003 mg mL^{-1}), linearity ($y = 2.8943x + 0.1386$, $r^2 = 0.9991$), precision and accuracy (95–104 %). Finally, the method was further applied to investigate the enantiomeric purity of levamlodipine in bulk samples.

Keywords Capillary electrophoresis · Levamlodipine · Enantiomeric purity · Carboxymethyl-β-cyclodextrin

Introduction

Amlodipine,3-ethyl-5-methyl-2-(2-amino-ethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate, is a potent di-hydropyridine calcium channel blocker used in the treatment of hypertension and angina pectoris [1–4]. The R- and S-enantiomer do not have the same biological activity [5, 6]. Only S-amlodipine (Fig. 1) posses vasodilation properties [7–9], whereas edema, headache, dizziness, facial flushing and other side effects come from the R-amlodipine [10–12]. Due to the significant pharmacokinetic difference between the two enantiomers and in order to improve the safety and efficacy, levamlodipine is produced as a single S-form and released to the market a few years ago [13, 14]. Therefore, R-enantiomer, as a chiral impurity, should be limited [15].

Several HPLC and HPCE methods have been developed for enantiomeric separation of *rac*-amlodipine [16–19]. The chiral separation of amlodipine enantiomers using vancomycin column or α1-acid glycoprotein-based stationary phase was reported by Gholami et al. and Lorenzi et al. [20]. Ansari et al. [21] reported a validated chiral LC method for the enantiomeric purity control of R-amlodipine on a Ultron ES-OVM chiral column and applied it on bulk drugs. Very recently, Majid et al. [22] developed a chiral CE method with polybrene as chiral selector for enantioseparation of amlodipine. To the best of our knowledge, there is no validated CE methods concerning the quality control of the R-amlodipine impurity of levamlodipine has so far been reported.

Capillary electrophoresis (CE) is an effective choice for resolving enantiomers and analyzing enantiomeric impurity [23–25] due to its high separation efficiency, ability to readily alter the nature and concentration of the chiral selector and low reagent consumption [26–28]. This paper reports the efforts in the development and validation of a CE method with CM-β-CD as chiral selector for the enantiomeric purity determination of levamlodipine. The buffer pH, chiral selector concentration, buffer concentration, capillary temperature and voltage were optimized. The aim of this work is to determine the enantiomeric impurity of levamlodipine at extreme values close to the

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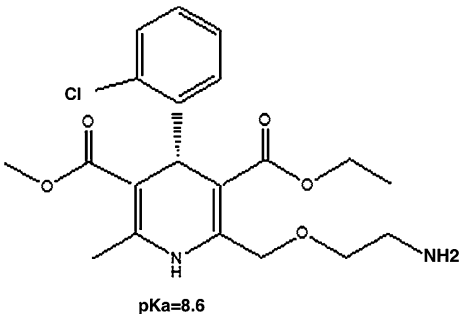

Fig. 1 Chemical structure and pKa value of levamlodipine

ICH recommendations [29] for the quality control of this raw material. Finally, the method was fully validated according to criteria imposed by the ICH guidelines.

Materials and Methods

Apparatus

All experiments were performed on a Beckman P/ACE MDQ capillary electrophoresis system (Fullerton, CA, USA), equipped with a diode-array detector for absorbance measurement. 32 Karat 8.0 Software (Beckman, Fullerton, CA, USA) was used for the instrumental control, data acquisition and data analysis. The temperature of the sample carousel was maintained by capillary cartridge coolant (Beckman, Fullerton, CA, USA).

Standards and Reagents

(R,S)-amlodipine besylate, S-amlodipine besylate and naloxone hydrochloride were purchased by National Institutes for Food and Drug Control (Beijing, China), R-amlodipine besylate was purchased by Chemsky international (Shanghai, China). Carboxymethyl-β-cyclodextrin was purchased by Sigma-Aldrich (CM-β-CD, DS = 3.5 per CD, USA). Methanol of chromatography grade was supplied by the Tianjin Concord Chemical Co. Ltd. (Tianjin, China). Sodium dihydrogen phosphate, sodium hydroxide, phosphoric acid and acetone of analytical grade were supplied by Shantou Xilong Chemical Co. Ltd. (Shantou, China). The levamlodipine besylate bulk drug was supplied by Liaoning Yicheng Pharmaceutical Co. Ltd. (Liaoning, China).

Electrophoretic Conditions

Electrophoretic separations were carried out with uncoated fused-silica capillaries (Ruifeng, Yongnian, Hebei, China) having a total length of 60 cm (effective length 50 cm) × 75 μm i.d. Prior to use, the new capillary was activated by flushing at 20 psi with 1.0 mol L^{-1} NaOH for 30 min and water for 10 min. At the beginning of each working day, the capillary was rinsed successively at 20 psi with water for 10 min, 0.1 mol L^{-1} NaOH for 20 min, water for 10 min and conditioned with BGE for 20 min. Between runs, the capillary was washed sequentially with 0.1 mol L^{-1} NaOH for 2 min, water for 2 min and the BGE for 3 min at 20 psi. At the end of each working day, the capillary was rinsed for 30 min with water and then dry stored.

Buffers and Standard Solutions

The BGE was prepared by dissolving the CM-β-CD in an appropriate concentration of NaH_2PO_4 buffer, and the desired pH adjustment was carried out with 0.1 mol•L^{-1} H_3PO_4 or 0.1 mol L^{-1} NaOH. Double distilled water was used throughout the study. The stock solutions of racemic amlodipine, S-amlodipine and R-amlodipine were prepared in methanol at a concentration of 2 mg mL^{-1}, and working solutions were prepared by diluting the stock solutions with water. The stock solution of naloxone (1 mg mL^{-1}) was prepared in water used as internal standard of the assay. All running buffers and sample solutions were filtrated with a 0.22 μm syringe type Millipore membrane and sonicated prior to use.

Results and Discussion

Optimization of Enantiomeric Separation

In this section, a mixed solution of racemic amlodipine and naloxone was injected at 90 and 10 ug mL^{-1}, respectively. The electroosmotic flow (EOF) was measured with 50 % acetone as neutral marker.

Effects of pH

The pH value of BGE is an important factor for the enantioseparation of charged analyte because it has direct influence on the EOF, the effective charge and mobility of the analyte. In this study, the influence of pH was investigated with the tested pH values controlled at 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0. As displayed in Table 1, the resolution as well as separation selectivity increased from pH 2.5 to pH 3.5, and then decreased as the pH increased from 4.0 to 5.0. In the investigated range of pH values, amlodipine was positive charged. When the pH increased from 2.5 to 3.5, the chiral selector, CM-β-CD, began to deprotonate and was negative charged gradually, and more importantly the

Table 1 Effect of buffer pH on the resolution (Rs), effective mobility (μeff), migration time (t) and separation selectivity (αeff)

pH	μ_{eo} (10^{-5}, $cm^2\ V^{-1}s^{-1}$)	$\mu_{eff\ R}$ (10^{-5},$cm^2\ V^{-1}s^{-1}$)	$\mu_{eff\ S}$ (10^{-5},$cm^2\ V^{-1}s^{-1}$)	$t_{(R)}$	$t_{(S)}$	α_{eff}	Rs
2.5	4.33	9.95	8.92	11.35	12.28	1.12	5.42
3.0	4.42	7.20	5.84	14.34	16.25	1.23	7.71
3.5	4.73	4.59	2.84	18.26	22.45	1.62	9.80
4.0	11.56	-0.40	-0.37	12.57	14.24	1.07	6.79
4.5	19.03	-1.94	-2.11	9.75	10.81	0.92	3.29
5.0	39.34	-19.59	-39.10	8.44	9.25	0.50	2.13

Separation condition: 4 mM CM-β-CD in 40 mM phosphate buffer at different pH. Voltage set at 30 kV, temperature set at 25 °C, hydrodynamic injection, 5 s at 20 psi; UV detection at 237 nm; normal polarity

EOF was very low under the pH 3.5. For the reasons above, the electrostatic interaction and the inclusion constants between chiral selector and analyte were strengthen, hence, the resolution and separation selectivity increased from pH 2.5 to 3.5. But when the pH increased from 4.0 to 5.0, there was a great growth on the EOF, the interaction time of CM-β-CD and analyte became shorter, consequently, the overall complexation between analyte and CM-β-CD became weaker, therefore the resolution and separation selectivity decreased as the pH increased. Finally, an optimal resolution of 9.80 was obtained at pH 3.5.

Effects of CM-β-CD Concentration

According to the capillary zone electrophoresis (CD-CZE) mathematical theoretical model system about enantiomeric separation established by Wren [30], "*Resolution changes show that there is an optimum concentration of chiral selector.*" The effects of CM-β-CD concentration were investigated in the range of 2.5–12 mM. The result is summarized in Fig. 2. Chiral resolution was achieved for all the concentrations within the range. With the increase of CM-β-CD concentration from 2.5 to 4 mM, an evident improvement of enantioseparation was observed, while the enantioseparation decreased when the CM-β-CD concentration change from 6 to 12 mM. The result was concordant with Wren's theory. This might result from the following aspects: higher CM-β-CD concentration would reduce the effective mobility, resulting in longer migration time and offering more opportunities for interactions between the analyte and CM-β-CD, which was beneficial to the complexation of the cyclodextrin with the analyte; but when CM-β-CD concentration was more than the theoretical optimal concentration, the differences in stability constants of temporary diastereomeric complexes reduced, even though the interaction time of the analyte and the selector became longer, and resultant the chiral resolution decreased. A CM-β-CD concentration of 4 mM was finally adopted as a compromise between resolution and run time.

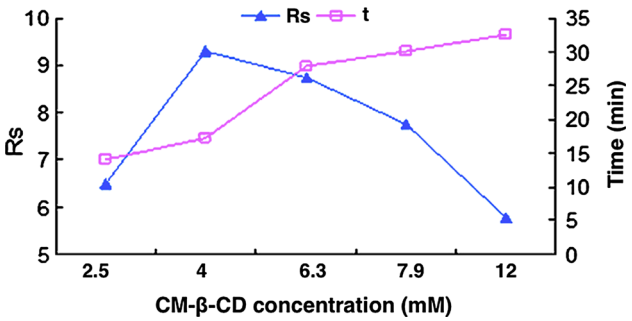

Fig. 2 Effect of CM-β-CD concentration on the resolution (Rs) and migration time (t). Separation condition: 40 mM phosphate buffer (pH 3.5), voltage set at 30 kV, temperature set at 25 °C; other conditions are the same as in Table 1

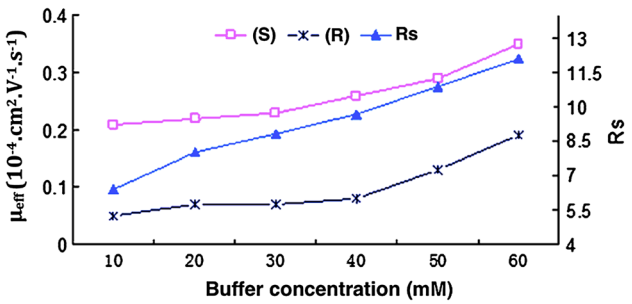

Fig. 3 Effect of buffer concentration on the resolution (Rs) and effective mobility (μeff). Separation condition: 4 mM CM-β-CD in different concentrations of phosphate buffer (pH 3.5), voltage set at 30 kV, temperature set at 25 °C; other conditions are the same as in Table 1

Effects of Buffer Concentration

Ionic strength of the buffer is also an important parameter for enantioseparation. The effects of different concentrations of phosphate buffer on the resolution (Rs) and effective mobility (μ_{eff}) of amlodipine were compared and presented in Fig. 3. An increase of phosphate concentration from 10 to 60 mM resulted in a distinct improvement of enantioseparation. This may be because that the increase of electrolyte concentration made the EOF decrease, due to narrowing of double-layer thickness at the capillary wall [31].

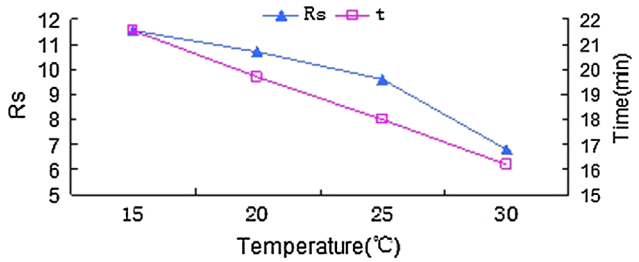

Fig. 4 Effect of temperature on the resolution (Rs) and migration time (*t*). Separation condition: 4 mM CM-*β*-CD in 40 mM phosphate buffer (pH 3.5), voltage set at 30 kV; other conditions are the same as in Table 1

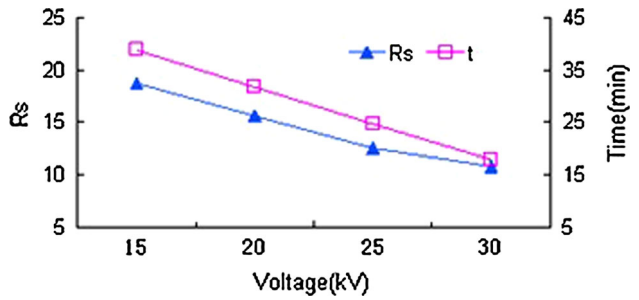

Fig. 5 Effect of the applied voltage on the resolution (Rs) and migration time (*t*). Separation condition: 4 mM CM-*β*-CD in 40 mM phosphate buffer (pH 3.5), temperature set at 25 °C; other conditions are the same as in Table 1

But as the phosphate concentration increased, the current and the baseline noise increased dramatically. Therefore, time spent on analysis became longer, but at the same time the resolution was higher. Taking all factors into consideration, the appropriate buffer concentration was set at 40 mM.

Effects of Temperature

The influence of temperature was also considered in the optimization process. Electrophoretic parameters were determined between 15 and 30 °C by 5 °C steps, and the result was illustrated in Fig. 4. The resolution reduced from 11.3 to 6.8, but the migration time decreased from 21.4 to 16.2 min when the temperature varied from 15 to 30 °C. Higher temperatures, by decreasing the buffer viscosity, could enhance the band broadening and thus decrease the resolution. Considering both run time and resolution, the temperature of 25 °C is suitable.

Effects of Voltage

In order to get a appropriate analysis time, the separation voltage was investigated at four voltages (15, 20, 25 and 30 kV), the enantioresolution increased as the voltage decreased with a longer migration time. The separation voltage was increased from 25 to 30 kV with a slight decrease of resolution (12.5 down to 10.8) but shorter migration time was observed in Fig. 5, so the applied voltage was choosed 30 kV. Thus, the optimal separation method was 4 mM CM-*β*-CD in 40 mM phosphate buffer (pH 3.5) with temperature and voltage set at 25 °C, 30 kV, respectively.

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