



Research Article

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Chiral separation of valsartan by CZE

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ABSTRACT

The capillary zone electrophoresis method for chiral separation of valsartan: complete separation of (R,S)-enantiomers of valsartan with a very good resolution ($R_s=1.64$) was achieved within an untreated fused silica capillary (75 μ m I.D., effective length was 60 cm). 30 mmol/L sodium acetate and 18 mg/mL β -CD (pH=4.50) was running buffer solution at the detection wavelength of 254 nm. Separation voltage was 20kv and the injection time of sample was 10 s. Results: The separation of both enantiomers of valsartan could be achieved baseline resolution. The calibration curve for valsartan optical isomers exhibited an excellent linear relationship in concentration range of 0.0625mg/mL -1.0mg/mL. The precision was less than 2%. The average recovery was 99.71% and 100.12%. In addition, The detection limit was 1.25 μ g/mL for valsartan enantiomer was 2.5 μ g/mL. Conclusion: The method is simple, rapid and accurate. It can be used to quality control the enantiomers of valsartan.

Keywords: Valsartan; Capillary Zone Electrophoresis; Chiral Separation

INTRODUCTION

Since there are differences in biological activity and toxicity between enantiomers, chiral resolution is an important subject in the development of the enantiomeric drugs [1]. A lot of chromatographic and spectroscopic methods have been developed for the analysis of enantiomers. Among the chromatographic methods so far developed, high-performance liquid chromatographic (HPLC) methods based on chiral enantioselective phases are widely employed for the assays of drug enantiomers in pharmaceutical preparations and biological fluids. However, one column can only separate a limited number of enantiomeric compounds. Recently, capillary electrophoretic (CE) methods using a chiral selector as the running buffer additive have been used for the above purposes. The chiral additives so far employed have included polysaccharides, proteins, bile salts and chiral mixed micelles. CD is a polysaccharides and include α , β , γ -CD[2-4].

Valsartan(3-methyl-2-[pentanoyl]-[4-[2-(2H-tetrazol-5-yl)phenyl]phenyl]methyl]amino]butanoic acid) is an angiotensin in commercial tablet's or capsules in different dosages as oral antihypertensive medication for patients whose high blood pressure is not adequately controlled on other component monotherapy[5]. Until now, high performance liquid chromatography has been the major technology used for the determination of the valsartan[6]. The structure of this basic drug is shown in Fig. 1.

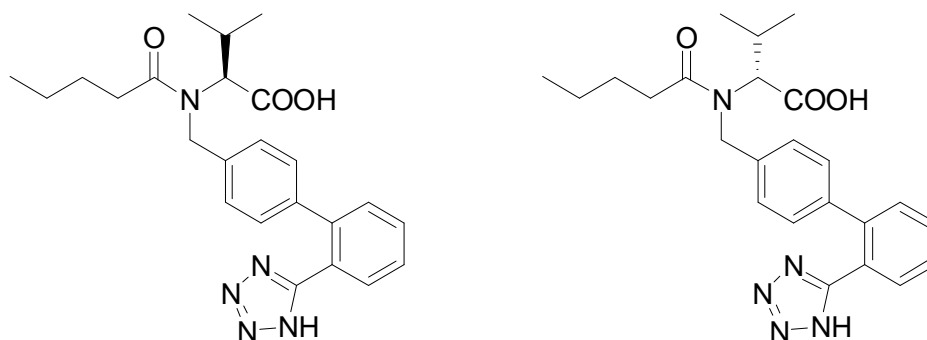


Figure1. Chemical structures of the valsartan isomers

In this study, The effects of concentrations of HP- β -CD, running buffer solutions, concentration and pH on the migration times and resolution of basic drug enantiomers were investigated to optimize the proposed method.

EXPERIMENTAL SECTION

2.1. Apparatus

The experiments were carried out on a laboratory-assembled CE apparatus, equipped with a multiwavelength UV detector. The UV signals were recorded at 270nm. An untreated fused silica capillary of 75 cm length (effective length 55cm) and of 75 μ m i.d. (Hebei Yongnian Optical Fiber Factory, China) was used as a separation tube. A high voltage power supply that can provide voltage from 0 to 30 kV was used to drive the separation.

2.2. Chemicals and reagents

Racemic valsartan was purchased from Hongxinkang Company. All organic solvents and other chemicals were of analytical grade. Hydrochloric acid (HCl), boric acid (H₃BO₃), Phosphate(H₃PO₄), Sodium dihydrogen phosphate (NaH₂PO₄), Sodium hydroxide (NaOH) and sodium acetate were obtained from Tianjin Chemical Reagent mainland. Triple-distilled water was used for the preparation of all solutions. 0.45 μ m pore size filters was used to filter all the solutions.

2.3. Sample preparation

About 10.0mg of valsartan racemic, accurately weighed, was transferred to a 10ml volumetric flask, and methanol was added to volume. The solution was filtered through a 0.45 μ m filter paper before injection.

2.4. Capillary electrophoresis

A new capillary was conditioned by flushing successively with 1.0 mol/l NaOH (overnight), 0.1mol/l NaOH (30 min) and then equilibrated with triple distilled water and running buffer each for 30 min before use. Between each injection, the capillary was rinsed with 0.1mol/l NaOH (2 min), triple distilled water (2 min) and with the running buffer (5 min)[3].

The conditions were as follows: sampling time, 10 s; applied voltage, 20 Kv; the detection wavelength, 254 nm. The experiments were carried out at room temperature. The electrolyte was a buffer solution consisting of 30mmol/L sodium acetate and 18mg/mL β -CD (pH = 4.50).

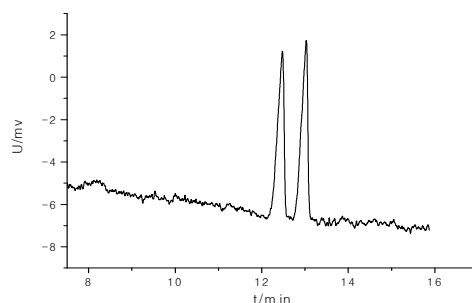


Figure 2. Electropherogram of the determination of valsartan in above CE conditions

RESULTS AND DISCUSSION

Different electrophoresis modes were tested in the test. The effect of buffer type, buffer concentration, buffer pH value, β -CD concentration, voltage were also investigated for the separation of valsartan by CE.

3.1 Analytical conditions

3.1.1 Effect of buffer type and concentration

The chemical composition and the concentration of the buffer can affect the baseline stability, peak shape and separation selectivity. In this study, the influence of buffer composition and concentration on the enantiomer separation of valsartan was investigated. The effects of the different buffers, NaH_2PO_4 , H_3BO_3 , NaAC on the enantiomer separation was showed in Fig.3. NaAC solution can give the excellent baseline enantiomeric separations for the valsartan. The effect of buffer concentration upon migration times and resolution was investigated in the range of 10-30mol/L. The enantiomer separation greatly improve with increasing concentration. Moreover, when the buffer concentration is 30mmol/L, the enantiomer separation is better.

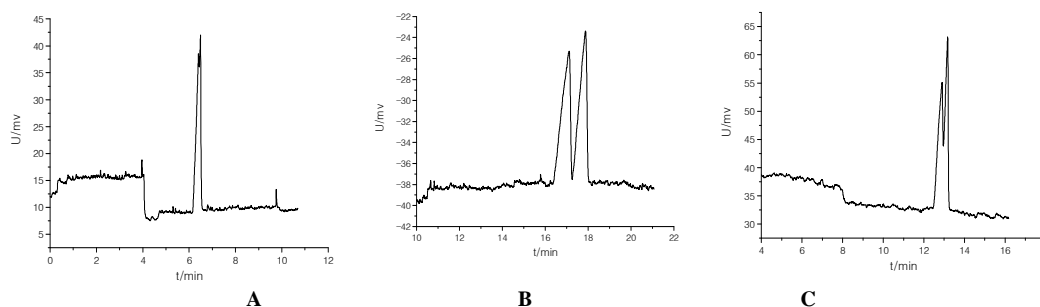


Figure 3. Electropherogram of the valsartan in different buffer solutions. A: NaH_2PO_4 B: NaAC C: H_3BO_3

3.1.2 Effect of pH

Valsartan molecules exist in two states of molecule (HA) and anion (A^-) in aqueous solution. β -CD can form two inclusion complexes with molecular valsartan enantiomers but not with ionic valsartan enantiomers. Therefore, pH can influence the distribution behavior of valsartan enantiomers.

The separation keep nearly unchanged at $\text{pH} < 3$, then increase rapidly with rising pH. But an opposite tendency is observed for enantioselectivity. This can be due to the fact that at low pH value ($\text{pH} < 3$) most extraction is through enantioselective complexation and at higher pH value ($\text{pH} > 3$) more nonselective partitioning of anion (A^-) is occurring. At $\text{pH} < 3$, valsartan molecules hardly dissociate, and the amount of molecule (HA) in aqueous phase is much bigger than that of anion (A^-). While with the further increase of pH ($\text{pH} > 3$) [5], more and more molecular valsartan enantiomers are dissociated into ionic valsartan enantiomers in aqueous phase, which leads to partitioning of more molecular valsartan enantiomers from organic phase to aqueous phase. Therefore, pH should be kept at 4.50 to carry out the extraction process.

3.1.3 Effect of β -CD concentration

CD is including α , β , γ -CD, of which α -CD is suitable for low molecular weight compounds, β -CD for medium-sized molecules, γ -CD for the larger molecular weight compounds. And valsartan is a soluble in water, medium-sized molecule, which is consistent with β -CD.

The concentration of β -CD for enantiomer separation was investigated. When the concentration of β -CD was 0mg/mL, enantiomer separation was 0. With an increase of the concentration of β -CD, the separation for valsartan enantiomers increased greatly. Moreover, when the concentration of β -CD was 18mg/mL, its separation was 1.64. However, as the concentration continues to increase to 60mg/mL or 70 mg/mL, the separation of enantiomer was decreased greatly. Therefore, the concentration of β -CD 18 mg/mL was selected .

3.1.4 Effect of Voltage

Finally, under the above selected conditions (18mg/mL β -CD concentration , 30mmol/L NaAC and pH4.50), the influence of the applied voltage from 10 to 25 kV was investigated. It can be observed that the increase in the separation voltage originated a decrease in the migration time and also in the resolution. However, a value of 20 kV gave rise to the shorter migration time (15 min) with enough resolution ($R_{1.64}$). Then, it was the separation voltage selected to obtain a rapid enantiomeric separation of valsartan enantiomers.

3.2 System suitability test

3.2.1. Linearity

In 0.10mg/mL ~ 0.50mg/mL range, five concentrations were collected, they were 0.10 mg/mL, 0.20 mg/mL, 0.25 mg/mL, 0.40 mg/mL, 0.50 mg/mL.

For S-valersartan, the correlation factor (r) is 0.9993 and the line equation is $Y=285229X+19325$; while for

R-valsartan, the correlation factor (r) is 0.9991 and the line equation is $Y=294987X+29920$, both of them indicating very good linearity.

3.2.2. Recovery studies

Recovery refers to the actual measured ratio of the amount and dosage. In the experiment, We need the preparation of a concentration of 80%, 100% and 120%. Each concentration was analyzed three times.

S-valsartan levoisomer average recovery was 99.71% and R-isomer was 100.12%. The results of standard addition recovery studies of S-valsartan R-isomer were shown in Table 1 and Table 2.

Table1 Assay results of recovery for S-valsartan

	Added/ (mg/ml)	Detected/ (mg/ml)	recovery/%	Average/%	RSD/%
80%	0.798	0.8	99.75	99.71	0.27
	0.796	0.8	99.50		
	0.799	0.8	99.88		
100%	0.996	1.0	99.60	99.57	0.22
	0.994	1.0	99.40		
	0.997	1.0	99.70		
120%	1.201	1.2	100.08	99.86	0.29
	1.198	1.2	99.83		
	1.196	1.2	99.63		

Table2 Assay results of recovery for R- valsartan

	Added/ (mg/ml)	Detected/ (mg/ml)	recovery/%	Average/%	RSD/%
80%	0.805	0.8	100.63	100.21	0.62
	0.802	0.8	100.25		
	0.798	0.8	99.75		
100%	1.007	1.0	100.70	100.27	0.64
	1.003	1.0	100.30		
	0.998	1.0	99.80		
120%	1.205	1.2	100.42	99.89	0.67
	1.197	1.2	99.75		
	1.194	1.2	99.50		

3.2.3. Detection limit and Limit of quantification

The detection limit is defined as the concentration that generates signal equivalent to three times noise ($S/N=3$). The detection limits for S-isomer and R-isomer are 1.25 $\mu\text{g}/\text{mL}$ and 2.5 $\mu\text{g}/\text{mL}$ respectively. The limit of quantification is defined as the concentration that generates signal equivalent to ten times noise ($S/N=10$). The limits of quantification for S-valsartan and R-isomer are 0.02 $\mu\text{g}/\text{mL}$ and 0.03 $\mu\text{g}/\text{mL}$ respectively.

3.2.4. Repeatability

Repeatability refers to the repeated determination of the measured values when the same volume levels consistent with each other. The relative standard deviations (RSDs) ($n=6$) of the peak area of S-valsartan and R-isomer were 1.58% and 1.35% respectively.

CONCLUSION

A rapid, simple, efficient and cheap method has been developed for the determination of valsartan at the same time by using high performance capillary electrophoresis method.

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