Chiral Separation of Two Pairs of Enantiomers of Tadalafil by High-Performance Liquid Chromatography

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Abstract

A high-performance liquid chromatographic method was developed for the chiral separation of a new selective phosphodiesterase 5 inhibitors, tadalafil and its three isomers. The chiral separation was performed on a Chiralpak AD column. The mobile phase was hexane–isopropyl alcohol (1:1, v/v). UV detection was at 220 nm. Baseline chiral separation for the four isomers was obtained within 30 min. Each of the resolutions of the two pairs enantiomers were more than 2.0. The limits of quantitation were 0.60, 0.90, 1.20, and 1.80 ng for (6R, 12aS), (6R, 12aR), (6S, 12aS), and (6S, 12aR) isomers, respectively. Relative standard deviation of the method was below 2% (n = 5). The method is suitable in quality control.

Introduction

Because of the existence of pharmacological and toxicological differences between enantiomers, chiral purity is now an integral part of drug research and development and of the regulatory process (1,2). For the analysis of enantiomers, a lot of chromatographic and spectroscopic methods have been developed. Among the chromatographic methods so far developed, high-performance liquid chromatographic (HPLC) methods based on chiral stationary phases are widely employed for the assays of drug enantiomers in pharmaceutical preparations and biological fluids (3,4). There were a few methods for the separation of two pairs of the enantiomers of one compound based on the same chromatographic system.

Tadalafil (6R-,12aR)-6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-methyl-pyrazino[1,2:1,6]pyrido[3,4-b]indole-1,4-dione (5), a new representative compound in the second generation of selective phosphodiesterase 5 (PDE-5) inhibitors, is a therapy used for the treatment of male penile erectile dysfunction (ED) and induces smooth muscle relaxation in the corpus cavernosum with onset around 30–45 min (6). ED is more common in advanced age (7–9) and may be related to some medical conditions (e.g., hypertension or diabetes mellitus) or use of certain pharmacological agents (e.g., antiarrhythmics and antihypertensive) (10). Drug testing is an integral part of pharmaceutical analysis and routine quality control monitoring of drug release characteristics. Therefore, pharmacokinetics and pharmacodynamics studies of tadaladil are required. Tadalafil shows five times more selectivity with respect to PDE-5 than sildenafil and vardenafil, which indicates inhibition of PDE-11 by Tadalafil at proper doses. It is a marked difference of tadalafil versus sildenafil and vardenafil, which are not expected to inhibit PDE-11 (11,12).

Tadalafil has two pairs of enantiomers, which are (6R, 12aS), (6R, 12aR), (6S, 12aS), and (6S, 12aR) isomers. Their molecule structures can be seen in Figure 1. But only (6R, 12aR) isomer is the pharmaceutical component according to primary studies (13). The others were regarded as chiral impurities. So far,

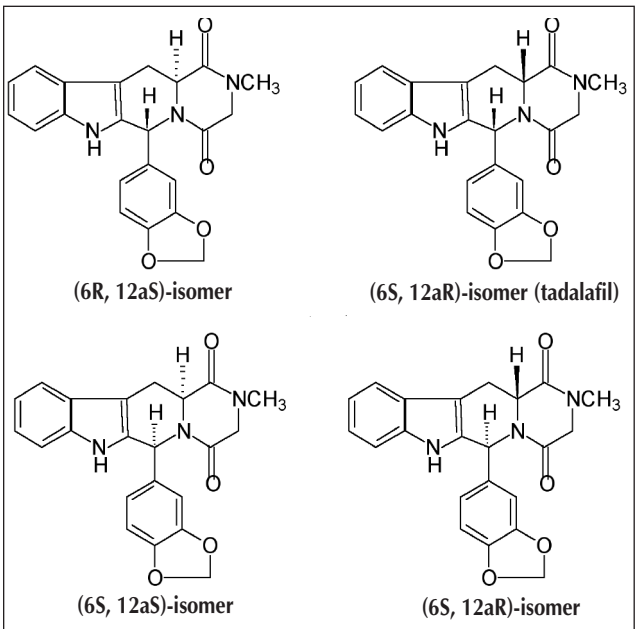

Figure 1. Chemical structures of two pairs of enantiomers of tadalafil.

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the separation of the two pairs of enantiomers was necessary. The methods for the determination of tadalafil were HPLC (5,14–17) and CE (18). These methods were for the determination of tadalafil and other compounds, but there is no separation method for the two pairs of enantiomers simultaneously reported to the best of our knowledge. In this paper, the aim was to develop a simple and precise HPLC method for the chiral separation and determination of the two pairs of enantiomers simultaneously.

Experimental

Materials

(6R, 12aS), (6R, 12aR), (6S, 12aS), and (6S, 12aR) isomers were supplied by BOERDA Pharmaceutical Company (Beijing, China). The recrystallization isomer was used as standard because commercial standard tadalafil were not obtained for us. The purity of recrystallization was more than 99% as determined by the HPLC method. Tadalafil tablet (20-mg tablet^{-1}) was supplied by BOERDA Pharmaceutical Company. *n*-Hexane (HPLC grade) was purchased from Shanghai Chemical Reagent Company (Shanghai, China). Isopropyl alcohol (IPA) (HPLC grade) and acetonitrile (HPLC grade) were purchased from ACROS (Geel, Belgium). Tetrabutylammonium hydroxide was purchased from Tianjin No. 1 Chemical Reagent Factory (Tianjin, China). All solutions and samples were filtered through a 0.45-µm membrane from Ruili Separation Instrument Company (Shanghai, China) before use. Eluent was composed of 40% *n*-hexane, 40% IPA, and 20% acetonitrile.

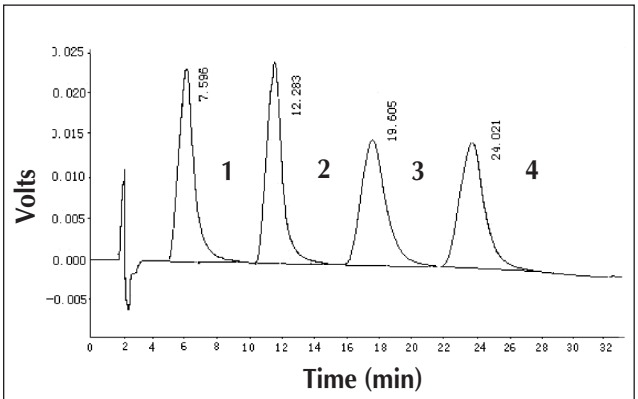

Figure 2. The chromatogram of the chiral separation. Sample solution contained each isomer 0.0125 mg/mL. Peak numbers are: Peak 1, (6R, 12aS) isomer; peak 2, (6R, 12aR) isomer; peak 3, (6S,12aS) isomer; and peak 4, (6S,12aR) isomer. Mobile phase: hexane–IPA (1:1, v/v).

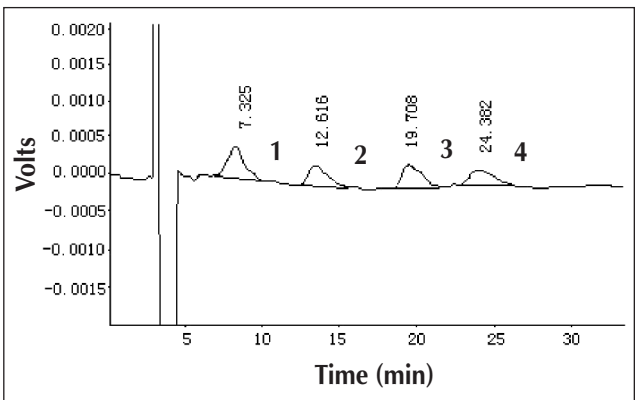

Figure 3. The chromatogram of the chiral separation. Sample solution contained each isomer 0.25 µg/mL. Peak numbers are: Peak 1, (6R,12aS) isomer; peak 2, (6R,12aR) isomer; peak 3, (6S,12aS) isomer; and peak 4, (6S,12aR) isomer.

Apparatus and conditions

The chromatography system consisted of JASCO (JASCO, Hachioji, Japan) PU-1580 and PU-1586 pumps, a variable wavelength UV-1570 detector. Data processing was carried out with a JASCO LC-1500 workstation. Chiralpak AD column packed with 10-µm silica gel coated by amylose *tris*-(3,5-dimethylphenylcarbamate) was from Daicel (Osaka, Japan). Hexane–IPA (v/v, 1:1) was used as the mobile phase. The flow rate was 0.75 mL/min, and 10 µL of the injection volume was used. The column temperature was kept at 30°C, and UV detection wavelength was 220 nm.

Solutions

Isomers (6R, 12aS), (6R, 12aR), (6S, 12aS), and (6S, 12aR) were weighed (12.5 mg, each) into a 200-mL volumetric flask, partially diluted with 150 mL of the diluent and sonicated for 5.0 min. Once dissolved, the solution was diluted to the volume with the diluent. Two milliliters of the solution were added into a 10-mL volumetric flask and diluted to the volume with the diluent. Tadalafil tablets were mashed and dissolved with the diluent for the same concentration. All the solutions were filtered before injection.

Results and Discussion

Chiral separation

The separation had been performed on different columns such as CHIRAL-AGP (100 × 4 mm, Chromtech), RESOLVOSIL BSA-7 (150 × 4 mm, Macherey-Nagel), CYCLOBOND II (250 ×

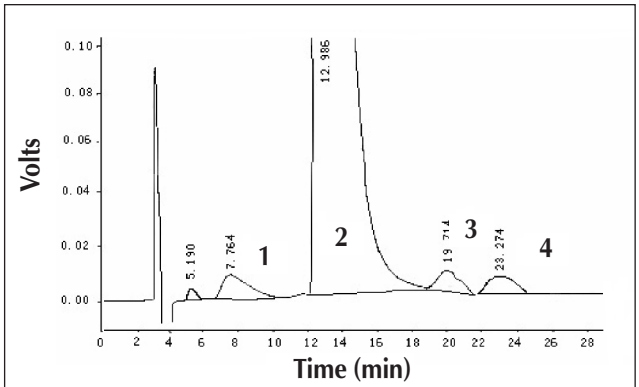

Figure 4. The chromatogram of the chiral separation. Sample solution contained (6R,12aR)-isomer 0.5 mg/mL and each the other isomer 0.25 µg/mL). Peak numbers are: peak 1, (6R,12aS) isomer; peak 2, (6R,12aR) isomer; peak 3, (6S,12aS) isomer; and peak 4, (6S,12aR) isomer.

4.6 mm, Astec), Chirobiotic R RISTOCETIN A (250 × 4.6 mm, Astec) and CHIRALCEL OC (250 × 4.6 mm, Daicel), but none of them could separate the two pairs of enantiomers at the same time for the experiment conditions. A method to separate all of them for quality control was needed.

Then, a Chiralpak AD column packed with 10-µm silica gel coated by amylose *tris*-(3,5-dimethylphenylcarbamate) was tested. From the experiments it was found that when IPA was used as the mobile phase, no chiral separation could be obtained with the column. When hexane was added to the mobile phase, the retention time of the sample became longer and the 6R, 6S-isomers began to be partly separated. The 6R, 6S-isomers were baseline separated when hexane (v/v) was greater than 40% in the mobile phase, but their enantiomers cannot be separated when the concentration of hexane was less than 45%. Resolution increased, and the enantiomers began to be separated with more hexane in the mobile phase. The two pairs of enantiomers were baseline separated within 30 min when 50% hexane was used in the mobile phase and the resolution of each pair enantiomers was over 2.0. However, the retention time increased greatly with the decrease of IPA in the mobile phase, so 50% was chosen as the optimum concentration of IPA. Figures 2 and 3 show the chromatogram of the sample solution containing 0.0125 mg/mL and 0.25 µg/mL for each isomer, respectively. From Figure 2, the retention factors of the four isomers and resolutions are shown in Table I. Figure 4 is a chromatogram containing 0.5 mg/mL (6R,12aR)-isomer and 0.25 µg/mL for the other isomers. Figure 5 is the chromatogram of the tadalafil tablet solution containing 0.5 mg/mL (6R, 12aR)-isomer sample. Figures 4 and 5 show that this method could determine the trace isomers in real samples.

The sample condition was also observed. It was shown in the experiment that the (6R, 12aR)-isomer could be completely converted to the (6R, 12aS)-isomer in strong basic conditions if one adds 1 mL of 0.1 mol/mL tetrabutylammonium hydroxide solution (in methanol) to 50 mL of 0.5 mg/mL (6R, 12aR)-isomer solution, mixes the samples, allows it to sit for 20 min, and then injects 10 µL of the described solutions into the HPLC. One can find that the (6R, 12aR)-isomer is completely converted to the (6R, 12aS)-isomer in the chromatogram. This indicates that tadalafil is not stabile in basic conditions.

Different flow rates and temperatures were examined. The results showed the temperatures have a low effect on the separation from 20–45°C, which was examined. The results of different flow rates from 0.5–1.0 mL/min showed that when the flow rate was greater than 0.75 mL/min, the back pressure was too high, and when the flow rate was less than 0.75 mL/min, the peaks would tail. The high pressure was caused by the high viscosity of the solution. So the flow rate was 0.75 mL/min, and the experimental temperature was 30°C.

Figure 6 shows the tablet sample separation chromatogram

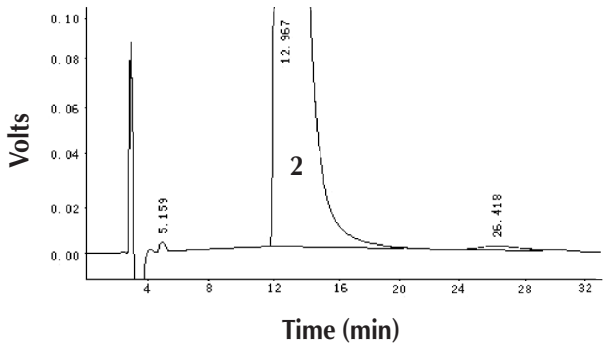

Figure 5. The chromatogram of the chiral separation. Sample solution contained (6R,12aR)-isomer 0.5 mg/mL. Peak 2 is the (6R,12aR) isomer.

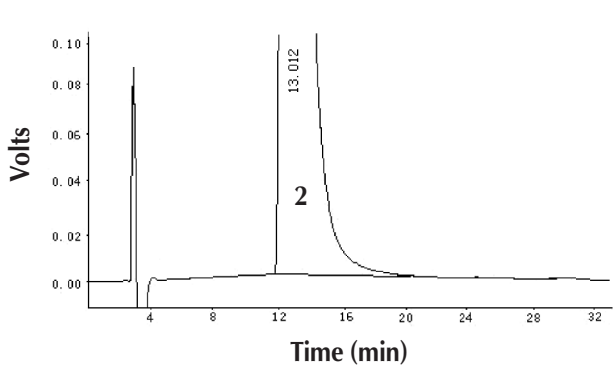

Figure 6. The chromatogram of the tablet. Tablet sample solution contained (6R,12aR)-isomer 0.5 mg/mL. Peak 2 is the (6R,12aR) isomer.

Table I. The Retention Factors (k′) and Resolutions (R)

	k′	R
6R,12aS	3.12	–
6R,12aR	6.61	2.52
6S,12aS	9.72	2.71
6S,12aR	12.05	2.14

Table II. Recoveries of the Four Isomers

Isomers	Amount added (µg)	Amount measured (µg)	Recovery (%)	Mean ± SD (%)
(6R,12aS)	2.78	2.84	102.20	100.60 ± 1.42
	3.48	3.47	99.7	
	4.18	4.17	99.8	
(6R,12aR)	2.86	2.94	102.8	101.2 ± 1.50
	3.58	3.62	101.1	
	4.30	4.29	99.8	
(6S,12aS)	2.92	2.95	101.0	99.5 ± 1.31
	3.65	3.60	98.6	
	4.38	4.33	98.9	
(6S,12aR)	2.80	2.74	97.9	98.8 ± 1.33
	3.50	3.51	100.3	
	4.20	4.12	98.1	

in the selected conditions. This demonstrates that the method was satisfactory in quality control.

Performance characteristics of the proposed method

Recovery

Three different amounts of the markers, 2.8, 3.5, and 4.2 µg for each isomer, were added to samples, respectively. The recoveries of the four isomers are listed in Table II. Each set of experimental data was an average of three duplicate injections.

Precision

The reproducibility (RSD) of the proposed method, on the basis of peak-area for five replications of (6R, 12aS), (6R, 12aR), (6S, 12aS), and (6S, 12aR) isomers, was 0.81%, 1.21%, 0.96%, and 1.32%, respectively. The RSD values of peak height for the four isomers were 1.24%, 1.38%, 1.77%, and 1.62%, respectively.

Calibrations and limits of detection

Calibration graphs were constructed in the range of 0.20–40 µg/mL for four isomers. The linear correlation equations of (6R, 12aS), (6R, 12aR), (6S, 12aS) and (6S,12aR) are:

$Y = 2.54 \times 10^7 X - 1.31 \times 10^2$ ($r = 0.9998$)

$Y = 2.51 \times 10^7 X - 1.02 \times 10^2$ ($r = 0.9997$)

$Y = 2.42 \times 10^7 X - 1.26 \times 10^2$ ($r = 0.9998$)

$Y = 2.36 \times 10^7 X - 1.13 \times 10^2$ ($r = 0.9996$)

The limits of quantitation were 0.60, 0.90, 1.20, and 1.80 ng, respectively. The limits of quantitation were obtained by a signal-to-noise ratio of 10.

Conclusion

From the previously given results, it is shown that the developed HPLC method based on a Chiralpak AD column for the chiral separation of two pairs of enantiomers of tadalafil was simple and sensitive. This method can be used as an effective technique for the quality evaluation of tadalafil.

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