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Chiral separation of Tamsulosin isomers by HPLC using cellulose Tris (3,5-dimethhylphenylcarbamate) as a chiral stationary phase

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Abstract

A high-performance liquid chromatographic (HPLC) method was developed for the chiral separation of an antagonist of alpha1A adrenoceptors, tamsulosin and its *S*-isomer. Baseline separation of the isomers was achieved within 35 min on a CHIRALCEL OD-RH column with a binary solvent mixture of 50 mmol l^{-1} KPF_6–acetonitrile (v/v (70:30), pH 5.0) as the optimized mobile phase. The detection limits and quantification limits of both *R*-isomer and *S*-isomer were 0.11 and 0.44 ng, respectively. The R.S.D. values of peak-area for the two isomer were 0.42% (of peak-height: 0.77%) for *R*-isomer and 0.64% (of peak-height:0.92%) for *S*-isomer ($n = 5$).

Keywords: Chiral separation; Tamsulosin (5-[(2R)-2-{[2-(2-ethoxyphenoxy) ethyl] amino}-propyl]-2-methoxy-benzenesulfonamide); Isomer; Cellulose tris (3, 5-dimethylphenylcarbamate) stationary phase

1. Introduction

Owing to the existence of pharmacological and toxicological differences between stereoisomers, chiral consideration is now an integral part of drug research and development and of the regulatory process [1,2]. Many 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 stationary phases are widely employed for the assays of drug isomers in pharmaceutical preparations and biological fluids [3–10].

Tamsulosin hydrochloride is used in the treatment of benign prostatic hypertrophy and was approved by the FDA in May 1997 after a review lasting 12 months [11]. The drug is a new antagonist of alpha1A adrenoceptors, which causes smooth muscle relaxation. Compared to other alpha-antagonists, tamsulosin has greater specificity for alpha1A receptors and does not affect alpha receptors on blood vessels [11,12]. Therefore, tamsulosin is less likely to cause hypotension compared to its analogs like prazosin [13]. Phase III clinical trials controlled by placebo found statistically significant improvements in Amer-

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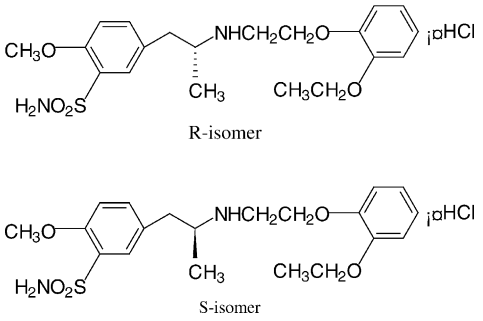

Fig. 1. Structures of (±)-5-[2(*R*,*S*)-2-{[2-(2-ethoxyphenoxy) ethyl] amino}-propyl]-2-methoxy-benzenesulfonamide monohydrochloride.

ican Urological Society efficacy parameters, such as symptom scores, after being treated with tamsulosin. The only side effects found in trials were abnormal ejaculation and dizziness [12–15].

(±)-5-[2(*R*,*S*)-2-{[2-(2-ethoxyphenoxy) ethyl] amino}-propyl]-2-methoxy-benzenesulfonamide are two isomers, but only *R*-isomer (tamsulosin) is the pharmaceutical component [11]. The molecular structure of the *R*- and *S*-isomer can be seen in Fig. 1. The analytical separation method of the two isomers has not been founded to be reported so far. The purpose of this paper is to develop a simple and precise method for the chiral separation of the *R*- and *S*- isomer by HPLC.

2. Experimental

2.1. Materials

Tamsulosin hydrochloride and its *S*-isomer were supplied by Huabei Pharmaceutical Company (Shijiazhuang, China). Acetonitrile (HPLC grade) and potassium hexafluorophosphate (analytical grade) were from Fisher (NJ, USA). All solutions and samples were filtered through a 0.45 μm membrane from Ruili Separation Instrument Factory (Shanghai, China) before used. Buffer and sample solutions were prepared by using deionized water obtained from a Milli-Q system (Millipore, USA).

2.2. Apparatus and conditions

The chromatography system consisted of JASCO (JASCO, Japan) PU-1580 and PU-1586 pumps, a variable wavelength UV-1570 detector. Data processing was carried out with a JASCO LC-1500 workstation. The columns such as CHIRAL-AGP(100 mm × 4 mm, Chromtech), RESOLVOSIL BSA-7 (150 mm × 4 mm, Macherey-Nagel), CYCLOBOND II (250 mm × 4.6 mm, Astec), Chirobiotic R RISTOCETIN A (250 mm × 4.6 mm, Astec), CHIRALCEL OC (250 mm × 4.6 mm, Daicel) and CHIRALCEL OD-RH (250 mm × 4.6 mm. Daicel) had ever been used in preliminary experiments. Chromatograghy was performed on the CHIRALCEL OD-RH column (250 mm × 4.6 mm, DAICEL CHEMICAL INDUSTAIES.LTD, Japan) packed with 5 μm silica gel coated by Cellulose tris(3,5-dimethylphenylcarbamate). 50 mmol l^{-1} KPF_6-acetonitrile (v/v (70:30)) was used as the mobile phase. The flow-rate was 1.0 ml min^{-1} and the injection volume was 10 μl. The detection wavelength was 225 nm. The column temperature was at 25–30 °C. Sample solutions were prepared by dissolving weighed tamsulosin (2.5 mg) and its *S*-isomer (2.5 mg) in 50 ml mobile phase.

3. Results and discussions

3.1. Chiral separation

In preliminary experiments, the separation had been performed on different columns such as CHIRAL-AGP(100 mm × 4 mm, Chromtech), RESOLVOSIL BSA-7 (150 mm × 4 mm, Macherey-Nagel), CYCLOBOND II (250 mm × 4.6 mm, Astec), Chirobiotic R RISTOCETIN A (250 mm × 4.6 mm, Astec) and CHIRALCEL OC (250 mm × 4.6 mm, Daicel), but none of them could separate the two enantiomers.

When Chiralcel OD-RH column (250 mm × 4.6 mm i.d.) was tested, the effects of mobile phase with different composition and concentration were investigated as the mobile phase also played an important role. Firstly, phosphate–acetonitrile mixture was used as mobile phase. By changing the concentration of acetonitrile and the pH value of the mobile phase, tamsulosin and its *S*-isomer could only be partly separated and the maximums resolution was 0.78 when the mixture was composed of (v/v (85:15)) phosphate buffer–acetonitrile with pH 2.0. Secondly, potassium

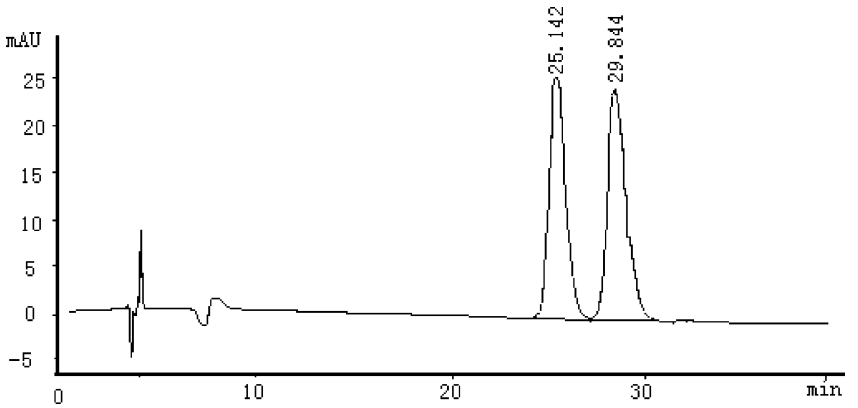

Fig. 2. Chromatogram of the sample solution containing each isomer 50 μg/ml. Peaks: 1, *R*-isomer; 2, *S*-isomer.

hexafluorophosphate (KPF_6) and acetonitrile mixture was tested for the separation. When pH value was kept at 4.5, the resolution was increased with increasing of acetonitrile concentration in the mobile phase. When the concentration of acetonitrile was 30%, the resolution could reach to 1.11. But when more acetonitrile was added in the mobile phase, the resolution become poor. So the binary mixture with (KPF_6)-acetonitrile (v/v (70:30)) was chosen in the following experiments. As the pH value of the buffer is a very important factor for chiral separation, when the mobile phase composition was fixed, the effect of pH value to the separation was investigated. From the experiments it was found that when pH was below 4.5 or above 6.1, the resolution of the enantiomers was below 1.1. When pH value was 5.0, the isomers could be baseline separated within 35 min, the resolution was 1.9 and the numbers of theoretical plate of the two isomers also reached a maximum. The chromatogram is shown in Fig. 2. So a binary solvent mixture of 50 mmol l^{-1} KPF_6-acetonitrile (v/v (70:30), pH 5.0) was chosen as the optimum separation condition for the isomers.

3.2. Power of the method

In order to illustrate the power of the method, a solution containing 2 mg ml^{-1} *R*-isomer and 2 μg ml^{-1} *S*-isomer were injected into the system. Under these conditions, *S*-isomer could be quantitated which showed that the method could quantitate 0.1% of the isomeric impurity besides the main component (see Fig. 3) and Fig. 4 is a chromatogram of tamsulosin hydrochloride, in which the concentration of tamsulosin hydrochloride product was 0.1 mg ml^{-1}.

3.3. Recovery

Three different marker concentrations: 2.03, 2.54 and 3.05 μg for *R*-isomer and 2.07, 2.59 and 3.11 μg

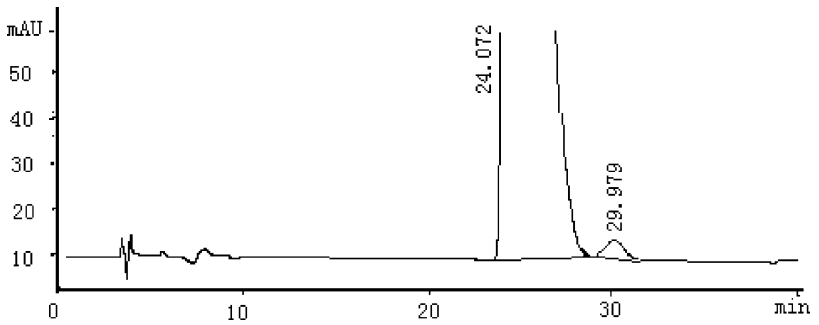

Fig. 3. Chromatogram of the sample solution containing *R*-isomer 2 mg ml^{-1} and *S*-isomer 2 μg ml^{-1}. Peaks: 1, *R*-isomer; 2, *S*-isomer.

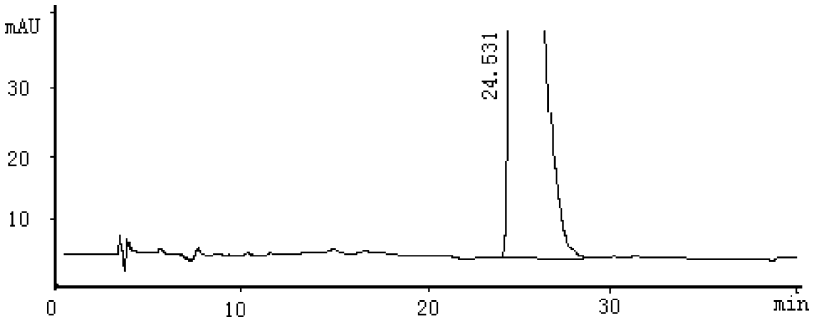

Fig. 4. Chromatogram of the sample solution containing 0.1 mg ml^{-1} of tamsulosin hydrochloride product.

Table 1
Recoveries of *R*- and *S*- isomer

Compound	Amount added (μg)	Amount measured (μg)	Recovery (%)	Mean ± S.D. (%)
R-	2.03	2.02	99.5	99.67 ± 2.55
	2.54	2.47	97.2	
	3.05	3.12	102.3	
S-	2.07	2.11	101.9	00.8 ± 1.96
	2.59	2.55	98.5	
	3.11	3.17	101.9	

for *S*-isomer were added to each sample solution. The recovery data of the two isomers were listed in Table 1. Each experimental data was an average of triplicate injections.

3.4. Precision

The repeatability (R.S.D.) of the proposed method, on the basis of peak-area for five replications, was 0.42% for *R*- and 0.64% for *S*-isomer. The R.S.D. values of peak height for the two isomers were 0.77% for *R*- and 0.92% for *S*-isomer. The R.S.D. values of retention times for *R*- and *S*-isomer were 0.84 and 0.89%, respetively.

3.5. Calibration

Calibration graphs were constructed in the range of 0.03–6.0 μg ml^{-1} for *R*-isomer and 0.028–5.6 μg ml^{-1} for *S*-isomer. The linear regression equations are: $Y = 1.35 \times 10^3 x - 1.86$ ($r = 0.9997$) and $Y = 1.48 \times 10^3 x - 2.41$ ($r = 0.9996$), respectively. The detection limits for the two isomers are 0.11 ng for *R*- and 0.13 ng for *S*-isomer. Detection limits were obtained by $S/N = 3$ with a sample solution containing single enantiomer.

4. Conclusions

From the above results, it can be seen that the proposed method is simple, sensitive and accurate, and can be used for the quality evaluation of tamsulosin hydrochloride.

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