Semipreparative Enantiomer Separation of Propranolol Hydrochloride by High-Performance Liquid Chromatography Using Cellulose *tris*(3,5-Dimethylphenylcarbamate) Chiral Stationary Phase

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Abstract

In this paper, we describe the direct semipreparative resolution of racemic (rac)-propranolol hydrochloride by high-performance liquid chromatography using cellulose *tris*(3,5-dimethylphenylcarbamate) as chiral stationary phase and mobile phase systems containing petroleum ether and 2-propanol with the use of basic additives. At analytical scale, the retention factor of both enantiomers is less than 5 with the separation factor 1.95 and the resolution 2.4. Then, the analytical method is scaled up to semipreparative loading to obtain small amounts of both propranolol hydrochloride enantiomers. Petroleum ether, rather than *n*-hexane, is used to effectively reduce the production cost of (*R*)- and (*S*)-propranolol. To increase the throughput, overlapping injections are used, allowing an injection to be made every 6.5 min instead of every 12 min. At semipreparative scale, approximately 19 mg/h enantiomers are isolated. The first fraction [(*R*)-(+)-propranolol hydrochloride] is isolated with a purity of > 99.6% (e.e.) and > 97.0% yield, and the second [(*S*)-(–)-propranolol hydrochloride] is isolated with a purity of > 99.3% (e.e.) and > 95.0% yield. In addition, optical rotations of both propranolol hydrochloride enantiomers isolated are investigated.

Introduction

Racemic (rac)-propranolol (Figure 1) is a β-adrenergic blocking agent. It is an optically active compound; however, it is usually given orally as a racemic mixture [i.e., 50:50 mixture of (*S*)-(–)-and (*R*)-(+)-enantiomers]. Propranolol hydrochloride has been found that the (*S*)-(–)-enantiomer is biologically active (1).

Many liquid chromatographic chiral methods have been used for the resolution of propranolol enantiomers. The enantiomeric resolution of propranolol hydrochloride can be obtained by using a direct method on polysaccharide-based chiral stationary phase (CSP) Chiralcel OD (2,3), cyclodextrin bonded column (4), Pirkle-type α-Burke 1 column (5), and α1-acid glycoprotein column (6). Karlsson (7) reported the resolution of propranolol hydrochloride using *N*-benzoxycarbonylglycyl-L-proline as chiral selector in the mobile phase; Fran Lai (8) reported the separation of propranolol hydrochloride using automated pre-column derivatization. Development of single isomer drug candidates has become standard practice in the pharmaceutical industry, research, and development laboratories. Direct chromatographic resolution on CSPs is considered today to be the most efficient for obtaining small amounts of both enantiomers with high optical purity in a short time, and has become an efficient tool in pharmaceutical research and early drug development (9,10).

Although some literature could be found which describes the direct preparative enantioselective chromatography of propranolol hydrochloride using supercritical fluid chromatography (11) and simulated moving bed technology (12), to the best of our knowledge, no paper has been published on the preparative resolution of enantiomers of propranolol hydrochloride by high-performance liquid chromatography (HPLC) on cellulose *tris*(3,5-dimethylphenylcarbamate) (CDMPC) CSPs using petroleum ether as the mobile phase. The aim of our work, therefore, is to develop a rapid, convenient, and economic method to prepare small amounts of both propranolol hydrochloride enantiomers to be used in pharmacological analysis and toxicological studies in a short time, which can be used easily in common laboratories.

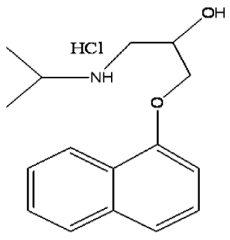

Figure 1. Chemical structures of (dl)-propranolol hydrochloride.

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Experimental

Instrumentation

The analytical HPLC system consisted of a Waters 441 HPLC pump, a UVIDEC-100-V detector (Jasco, Tokyo, Japan), and a 7125 syringe loading sample injector (Rheodyne, Rohnert Park, CA) equipped with 20-µL loop. The chromatographic data were acquired and processed by HW-2000 chromatography manager software model.

The preparative HPLC system consisted of a G1361A pump (Agilent, Palo Alto, CA), a G1315B diode array and multiple wavelength detector (Agilent), G1364 fraction collectors (Agilent), and a 7125-083 syringe loading sample injector (Rheodyne) equipped with 1000-µL loop. The chromatographic data were acquired and processed by Agilent 1100 chromatographic manager software model.

Optical rotations were measured on a polarimeter Perkin-Elmer model 341 equipped with a Na lamp, at the wavelength of 589 nm. The volume of the measuring cell was 5 mL and the optical path was 10 cm. The system was thermostated at 20°C.

Materials

Microcrystalline cellulose was purchased from the Fourth Reagent Factory of Shanghai (Shanghai, China); 3,5-dimethylphenylisocyanate was obtained from Aldrich (St. Louis, MO). 3-Aminopropyltriethoxysilane was a product of Liaoning Chemical Plant (Liaoning, China). The spherical silica gel (with a mean particle size of 5 µm, a mean pore diameter of 27 nm, and a specific surface area of 59 m^2/g) was made in our laboratory. All other reagents used were of analytical grade from Tianjin Second Chemical Reagent Plant (Tianjin, China). The propranolol hydrochloride was purchased from Linfen Yunpeng Medicinal Corporation.

Chromatographic conditions

The size of the analytical column was 5 cm × 4.6 mm and 15 cm × 4.6 mm, and the preparative column was 15 cm × 11.5 mm. Mobile phases were filtered and degassed in an ultrasonic bath before use. The column temperature was ambient temperature. The flow rates were 1.0 and 6.25 mL/min for analytical and semipreparative HPLC, respectively. The detection wavelength was 290 nm. Propranolol hydrochloride was prepared by dissolving it in methanol and filtered before use.

Every value was repeated three times. The dead time (t0) was determined using the first solvent chromatogram. The separation factors (α) were calculated as $\alpha = k_2/k_1$ where k_1 and k_2 were retention factors for the first and second eluting enantiomer, respectively. The resolutions (Rs) were calculated by the following formula: $Rs = 1.18(t_2 - t_1) / (w_{(1/2)1} + w_{(1/2)2})$, where $w_{(1/2)1}$ and $w_{(1/2)2}$ were half-peak widths for the first and second eluting enantiomer, respectively.

For the coating amount, $\%(w/w) = (w_{CDMPC} / w_{CSP}) \times 100\%$, w_{CDMPC} and w_{CSP} were the mass of CDMPC and CSP, respectively. Recovery % = the ratio of the peak area of the fraction collected versus the peak area of the same fraction in the sample. Purity (e.e.) % of *R*-propranolol = [(*R*) – (*S*)] / [(*R*) + (*S*)] where (*R*) or (*S*) was the chromatographic peak area of *R*- or *S*-propranolol.

Preparation of chiral stationary phase

The aminopropylated silica gel (APS) was prepared as described in reference 13, elemental analyses (%): C, 1.476; N, 0.323; H, 0.396. CDMPC was prepared as described in reference 14, elemental analyses (%): C, 64.55; N, 6.950; H, 5.790. (Calculated %: C, 65.66; N, 6.960; H, 6.180).

An appropriate amount of CDMPC was dissolved in 30 mL of tetrahydrofuran. APS (1 g) was stirred in tetrahydrofuran (10 mL) at 40°C. The CDMPC solvent (10 mL) was added until the solvent was near dryness. The solvent was removed under stirring at 40°C to near dryness, and this was repeated twice. The CSP was finally dried under vacuum.

The prepared CSP was packed into a stainless steel column (5 cm × 4.6 mm and 15 cm × 4.6 mm for the analytical column, 15 cm × 11.5 mm for the semipreparative column) by the conventional high-pressure slurry-packing procedure.

The preparation of columns of this type has been shown to be very reproducible from one batch to another (e.g., several batches of CDMPC-coated APS supports gave very similar α and Rs values for a series of our test analytes).

Results and Discussion

Choice of the chiral stationary phases

The CDMPC-CSP is one of the best chiral stationary phases for the separation of propranolol hydrochloride racemate (2) and has usually been used in laboratories. It has a high loading capacity, which is important for preparation chromatography. We selected CDMPC as the chiral stationary phase, which can satisfy the preparative separation and can be obtained easily in common laboratories.

The CDMPC has usually been coated onto a macroporous APS support (15). The polysaccharide with large molecular weight is more suitable to be coated on large pore size, so we chose the support with larger pore size (27 nm).

The previous studies (16) have shown that polysaccharide tris(aryl carbamate) derivatives coated onto large pore (1000–4000 Å, 7–10 µm) silica gel have the best separation performance with the large coating amount of 20% (w/w). But our experience has told us that the coating amount of the CSP which has the best separation performance was not the same value due to the difference of pore size and surface area of the support. So we firstly investigated the influence of coating amount upon RS, k_2, and α on APS by the resolution ability of propranolol hydrochloride, and the results were shown in Figure 2.

Figure 2 shows that a coating of 11–17% (w/w) CDMPC is optimum for the support with 27 nm pore size. At low levels (< 11%, w/w), an increase in the carbamate loading was accompanied by an increase in the capacity and separation factors. This was assumed to be due to an increase in the number of carbamate interaction sites. However, as the loading was further increased, the resolution of the sample did not increase any more because the penetrability of the sample on the CSP became worse and the peak of the sample became wider as the coat of the CDMPC became thicker. When the coating amount > 17%, the resolution deteriorated and eventually the part became aggre-

gated and would not pack satisfactorily. We chose the CDMPC-CSP with a comparatively small coating amount of 11%, considering the production cost of this method.

The use of those shorter, high-resolution columns will typically reduce solvent consumption and result in more concentrated fractions. So we packed the CSP into a stainless steel column (15 cm × 4.6 mm for the analytical column and 15 cm × 11.5 mm for the semipreparative column) by the conventional high-pressure slurry-packing procedure. The test of the column is shown in Table I. In Table I, it is shown that the analytical chromatography could be scaled up to preparative chromatography satisfactorily. The semipreparation system had less dead volume than that of the analytical system, so the resolutions of the preparative column were even better than that of the analytical column.

Analytical HPLC

Our first effort was to resolve a racemic mixture of propranolol hydrochloride under different elution conditions. From Table II, it can be seen that good enantioselectivity and resolution of propranolol hydrochloride were obtained with the CDMPC-CSP using normal mobile phases.

Baseline separation on CDMPC-CSP was achieved by using either *n*-hexane or petroleum ether as the mobile phase. We tried to direct our experiments aiming the development of analytical methods that could be scaled up to preparative loadings. The petroleum ether is much cheaper than *n*-hexane. And it can be seen in Table II that the use of petroleum ether could replace *n*-hexane completely. Thus it could be anticipated that petroleum ether is more suitable than *n*-hexane as the mobile phase for the preparative resolution of propranolol hydrochloride enantiomers.

The addition of DEA in the mobile phase can improve the resolution of propranolol hydrochloride. DEA is considered to improve consequent effects on peak shape and Rs.

Up to date, the chiral discrimination mechanism of polysaccharide phases has been assumed that the separation of racemates on amylose- and cellulose-based CSPs was due to the formation of solute-CSP complexes through inclusion of the enantiomers into the chiral cavities in the higher order structures of the CSPs. Chiral discrimination between the enantiomers is due to the differences in their steric fit in the chiral cavities (14,17). In the CSPs with carbamate derivatives, the binding of the solutes to the CSPs was achieved through interactions between the solutes and the polar carbamate groups on the CSPs (14,17). In this study, π-π interactions may be important between the naphthyl rings of solutes and the phenyl rings of the CSPs in chiral recognition. In addition, the hydrogen bond interactions between –OH, –O– of the solutes and the polar carbamate groups on the CSP may be also important in the discrimination.

When developing analytical methods for scale-up to preparative, it is desirable to have an analytical resolution greater than 2 and k for the desired compound of less than 5 (18). Petroleum ether–2-propanol–DEA (85:15:0.1, v:v:v) was selected as the mobile phase of semipreparative HPLC of propranolol hydrochloride enatiomers in this study. Using this mobile phase, better resolution (Rs > 2), low retention factor (k_2 < 5), and the best column efficiency could be obtained due to the most narrow peak shape and least tailing. The chromatograms of the chiral

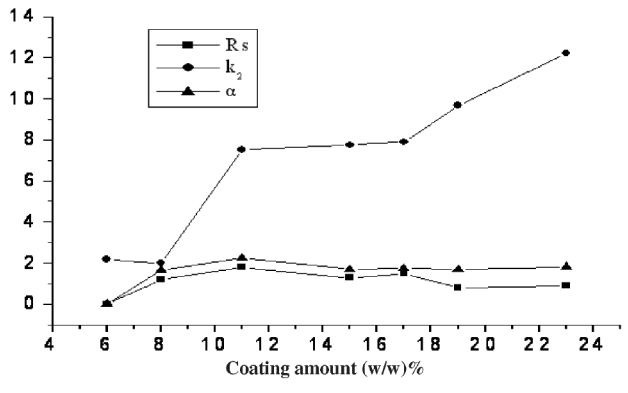

Figure 2. Influence of coating amount upon Rs, k_2, and α. Column CDMPC-CSP (5 cm × 4.6 mm); flow rate: 1.0 mL/min; mobile phase: *n*-hexane–2-propanol = 90:10 (v:v); detection (UV): 290 nm.

Table I. The Test of the Column*

Column	Injection amount (mg)	k_2	Rs	α	Plates/m
Analytical †	0.002	4.223	2.4	2.36	2653
Semipreparative‡	0.395	5.260	2.7	2.17	1973

* Mobile phase: petroleum ether–2-propanol–DEA = 85:15:0.1 (v:v:v), detection (UV): 290 nm.
† Column CDMPC-CSP (15 cm × 4.6 mm) coating amount of 11% (w/w), flow rate: 1 mL/min.
‡ Column CDMPC-CSP (15 cm × 11.5 mm) coating amount of 11% (w/w), flow rate: 6.25 mL/min.

Table II. Chromatographic Results of the Chiral Separation of Propranolol Hydrochloride in Various Mobile Phases*

Mobile phase	Volume ratio	k_2	Rs	α
Methanol	100	0.287	–	–
Ethanol	100	0.418	–	–
2-propanol	100	0.502	–	–
Petroleum ether–2-propanol	50:50	1.200	0.9	1.48
Petroleum ether–2-propanol–DEA	50:50:0.1	1.154	1.1	1.52
Petroleum ether–2-propanol–DEA	50:50:0.2	1.099	1.1	1.54
Petroleum ether–2-propanol–DEA	70:30:0.1	1.872	1.5	1.64
Petroleum ether–2-propanol–DEA	80:20:0.1	2.967	2.1	1.77
Petroleum ether–2-propanol–DEA	85:15:0.1	4.223	2.4	1.95
n-Hexane–2-propanol–DEA	85:15:0.1	4.067	2.3	1.89
Petroleum ether–2-propanol–DEA	90:10:0.1	6.089	2.5	2.02

* Column CDMPC-CSP (15 cm × 4.6 mm i.d.) coating amount of 11% (w/w), flow rate: 1 mL/min; detection (UV): 290 nm.

separation of propranolol hydrochloride on the analytical column are shown in Figure 3.

Semipreparative HPLC

The flow rate used for the larger column should ensure the same linear velocity of mobile phase as used in the analytical run. In this experiment, a flow rate of 6.25 mL/min was calculated by the following formula for the semipreparative HPLC: $V_1/V_2 = (r_1/r_2)^2$ (19) where r_1 and r_2 are the diameter for analytical and preparative column, respectively; V_1 and V_2 are the flow rate for analytical and preparative HPLC, respectively.

In preparative HPLC, loading capacity is one of the critical factors. It indicates the maximum amount of racemate that the column can tolerate without compromising resolution. Therefore, it has a determining impact on throughput. In this paper, a concentration of 7.9 mg/mL was selected for the semipreparative resolution of propranolol hydrochloride racemate. Semipreparative chromatogram of propranolol hydrochloride is shown in Figure 4 and the impact of the injection volume on the resolution is shown in Table III. As the injection volume was further increased, the dissolution of the sample in mobile phase became not very well. The peaks of the sample became wider due to the slow dissolution of the sample in mobile phase. We can see from Table III that throughput (the amount produced per injection) of individual enantiomers (e.e. > 99%) decreased with the increase of injection volume.

It can be seen in Figure 4 that there is still some distance between two enantiomer peaks and the loading capacity is less than the maximum value that the column can tolerate. A relatively low value of loading capacity was determined mostly due to the tailing of the first peak being delayed into the second peak, which directly influenced the purity of the second individual enantiomers isolated. We determined the injection volume of 250 µL, which allowed a good compromise between throughput and purity. To enhance throughput, the technique of overlapping injections (also called boxcar injection) and it is not necessary to wait until all components elute from the column. Using this

Table III .The Effect of Injection Volume on the Recovery and RS*

Injection volume (µL)	50	100	200	250	300
RS	2.69	2.40	2.40	2.30	2.21
Recovery% 1†	99.9	99.3	99.3	97.3	97.3
Recovery% 2†	99.0	99.2	98.7	95.0	84.3
RSD% of recovery 2	0.25	0.40	0.50	2.5	8.0

* Column CDMPC-CSP (15 cm × 11.5 mm i.d.) coating amount of 11% (w/w); mobile phase: petroleum ether–2-propanol–DEA = 85:15:0.1 (v:v:v), flow rate: 6.25 mL/min; detection (UV): 290 nm.
† Purity of the fraction isolated > 99%.

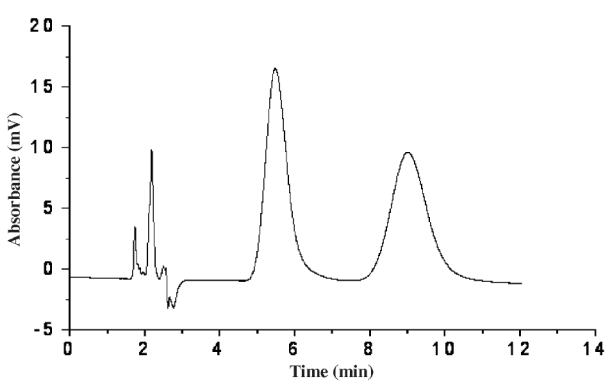

Figure 3. Chromatogram of the chiral separation of propranolol hydrochloride racemate on analytical column. Column CDMPC-CSP (15 cm × 4.6 mm, i.d.) coating amount of 11% (w/w); mobile phase: petroleum ether–2-propanol–DEA = 85:15:0.1 (v:v:v); flow rate: 1 mL/min; detection (UV): 290 nm.

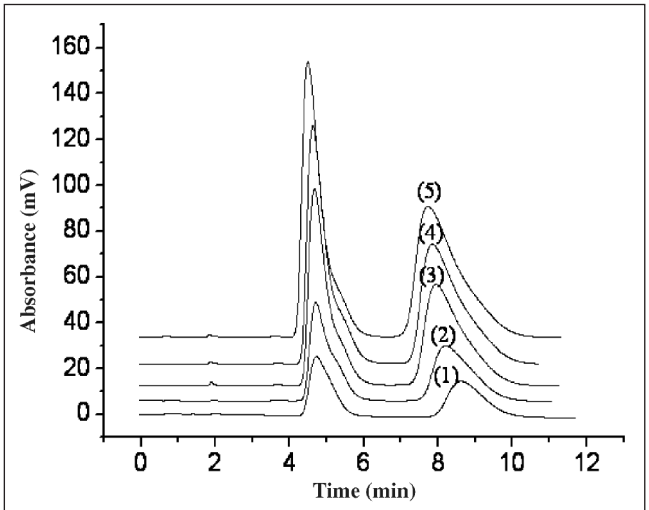

Figure 4. Semipreparative chromatogram of propranolol hydrochloride. Injection volume: 50 µL (peak 1); 100 µL (peak 2); 200 µL (peak 3); 250 µL (peak 4); 300 µL (peak 5). Column CDMPC-CSP (15 cm × 11.5 mm i.d.) coating amount of 11% (w/w); mobile phase: petroleum ether–2-propanol–DEA = 85:15:0.1 (v:v:v); flow rate: 6.25 mL/min; detection (UV): 290 nm.

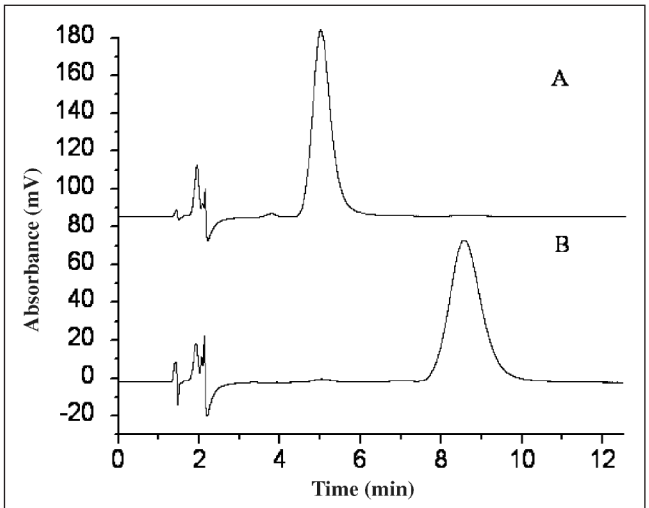

Figure 5. Chromatogram of the propranolol hydrochloride enantiomers isolated reinjected in the analytical column: the first fraction isolated (A), the second fraction isolated (B). Column CDMPC-CSP (15 cm × 4.6 mm i.d.); mobile phase: petroleum ether–2-propanol–DEA = 85:15:0.1 (v:v:v); flow rate: 1 mL/min; detection (UV): 290 nm.

technique can effectively use the time between the injection and the first enantiomer eluted to make another injection, and it allowed an injection to be made every 6.5 min instead of every 12 min.

At semipreparative scale, approximately 19 mg/h enantiomers could be isolated. Nine-point-five milligrams of the first fraction and 9.2 mg of the second fraction were isolated in an hour. The chromatograms are shown in Figure 5. The first eluting enantiomer was isolated with a purity of 99.6% (e.e.) and a yield of > 97.0%, and the second eluting enantiomer was isolated with a purity of 99.3% (e.e.) and a yield of > 95.0%. In this experiment, the isolated enantiomers were dried, sealed, and kept in room temperature. Two weeks later, the e.e. values of the fractions were determined again and almost the same values (99.4% for the first fraction and 99.0% for the second fraction) were obtained.

The optical rotations of two enantiomers collected were also measured. The isolated enantiomers were characterized as follows: the first fraction isolated, (*R*)-(+)-propranolol hydrochloride: e.e. = 99.6%, $[\alpha]^{20}_D$ = + 16.3°(c = 1.96, ethanol); the second fraction isolated, (*S*)-(–)-propranolol hydrochloride: e.e. = 99.3%, $[\alpha]^{20}_D$ = –16.2°(c = 1.54, ethanol).

Conclusions

In this paper, a simple, rapid, and economic preparative method was developed for the chiral separation of propranolol hydrochloride racemate on CDMPC chiral stationary phase using petroleum ether–2-propanol–DEA (85:15:0.1, v:v:v) as the mobile phase. This method will show results easily using an HPLC in laboratories, and does not need a professional preparative HPLC. The use of cheaper petroleum ether instead of *n*-hexane reduced the production cost of the individual propranolol hydrochloride. Small quantities of high purity (> 99%) of both propranolol hydrochloride enantiomers were obtained in a short time. The throughput and the purity of the production can satisfy the need of pharmacological analysis and toxicological studies in laboratory level.

Acknowledgment

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