High performance liquid chromatographic separation of thirteen drugs collected in Chinese Pharmacopoeia 2010 (Ch.P2010) on cellulose ramification chiral stationary phase

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Abstract The enantiomers separation of thirteen drugs collected in Ch.P2010 was performed on chiral stationary phase of cellulose ramification (chiralpak OD and chiralpak OJ) by high performance liquid chromatographic (HPLC) methods, which included ibuprofen (C1), ketoprofen (C2), nitrendipine (C3), nimodipine (C4), felodipine (C5), omeprazole (C6), praziquantel (C7), propranolol hydrochloride (C8), atenolol (C9), sulpiride (C10), clenbuterol hydrochloride (C11), verapamil hydrochloride (C12), and chlorphenamine maleate (C13). The mobile phase consisted of isopropanol and n-hexane. The detection wavelength was set at 254 nm and the flow rate was 0.7 mL/min. The enantiomers separation of these thirteen racemates on chiralpak OD column and chiralpak OJ column was studied, while the effects of proportion of organic additives, alcohol displacer and temperature on the separation were studied. And the mechanism of some of racemates was discussed. The results indicated that thirteen chiral drugs could be separated on
1. Introduction

Biggish differences exist among optical enantiomers of chiral drugs in pharmacodynamics and toxicity. It is mainly due to the fact that receptors and enzymes possess special stereoselectivity to different enantiomers of drugs in the process of absorption, distribution, metabolism and elimination. Therefore, the studies on pharmacokinetics and pharmacodynamics for the single enantiomer should be explored to ensure drugs to be reasonable, safe and reliable in the clinical usage [1,2]. To guarantee the quality of the enantiomer for the pharmacological research, an analytical method of separation for drug enantiomers is indispensable to establish [3]. Up to now, the separation methods for chiral drug have been reported on chemical separation, crystallization method, biological separation, chromatography method, etc. [4]. It has been indicated that chromatography could be deemed as the best effective way based on simplicity, convenience, and accuracy. Although cellulose chiral columns were applied to separate some racemates and enantiomers, such as nitrendipine (C3) enantiomers and the separation mechanism was of diethylamine–n-hexane–isopropanol. The flow rate was 0.7 mL/min and the injection volume was 10 μL. The analytes were monitored at 254 nm.

2. Experimental

2.1. Chemicals and materials

All reagents in the experiment were purchased from Merck. Besides n-hexane, isopropanol and diethylylamine were of HPLC grade, the others were of analytical grades. Solvents were filtered through 0.22 μm membrane filters before analysis.

The reference standards of ibuprofen, ketoprofen, nitrendipine, nimodipine, felodipine, omeprazole, praziquantel, propranolol hydrochloride, atenolol, sulpiride, clenbuterol hydrochloride, verapamil hydrochloride, chlorphenamine maleate were supplied from National Institutes for Food and Drug Control (Beijing, China).

2.2. Preparation of standard solutions

Each reference was accurately weighed and dissolved in isopropanol as the stock solutions (1 g/L). All the solutions were stored at 4 °C in refrigerator. A series of standard solutions were prepared by dilution of the stock solution with the mobile phase before injection.

2.3. Analytical method

A Shimadzu LC-10A TVP HPLC system was equipped with SCL-10A TVP central controller, LC-10A TVP quaternary pump, CTO-10 AVP column heater-cooler, SIL-10A DVP vacuum degasser autosampler, SPD-10 AVP UV detector, and LC solution chromatography data workstation.

The chromatographic separation was performed on Chiralcel OJ-H (250 mm × 4.6 mm, i.d., 5 μm) and Chiralpak OD-H (250 mm × 4.6 mm, i.d., 5 μm). The mobile phase consisted of diethylylamine–n-hexane–isopropanol. The flow rate was 0.7 mL/min and the injection volume was 10 μL. The analytes were monitored at 254 nm.

3. Results and discussion

3.1. Investigation of chromatographic action on cellulose chiral columns

Both chiralpak OD and chiralpak OJ belong to cellulose chiral columns possessing the chiral functional groups of tris (3,5-dimethylphenylcarbamate) or tris (4-methylbenzoate) coated on silica surface. They have strong selectivity to some chemical structures with chiral centers. Its carbonyl in the structure could have dipole-dipole action to some target
compounds with carbonyl, or invoke hydrogen bonding with some compounds with hydroxyl. Its phenyl in the chemical structure would produce \( \pi - \pi \) action by aromatic compounds [8].

3.1.1. Chiral separation on Chiralcel OD-H
Nimodipine (C4), omeprazole (C6), praziquantel (C7) and propranolol hydrochloride (C8), and atenolol (C9) performed a well separation with a pair of enantiomers on OD-H column, while sulpiride (C10) was not separated with the resolution value (Rs) of 0.9. The resolution of sulpiride (C10) was not improved significantly by adjustment of chromatographic conditions. Five types of racemates such as nitrendipine (C4), felodipine (C5), clenbuterol hydrochloride (C11), verapamil hydrochloride (C12) and chlorphenamine maleate (C13) were eluted as a single peak indicating they could not be separated on OD-H column. No peak was detected when ibuprofen (C1) or ketoprofen (C2) was injected into HPLC on the same column. The results are shown in Table 1 and the HPLC chromatograms of C4, C6, C7, C8 and C9 are displayed in Figs. 1–5.

3.1.2. Chiral separation on Chiralcel OJ-H
The chromatographic separation on Chiralcel OJ-H column was very different from that on Chiralcel OD-H column for the same sample. ibuprofen (C1), ketoprofen (C2), felodipine (C5), omeprazole (C6), clenbuterol hydrochloride (C11), and chlorphenamine maleate (C13) were able to be separated completely with a pair of peaks of enantiomers. Nitrendipine (C3) and nimodipine (C4) can be separated with a resolution value of 0.7 or 1.2. The bifurcation phenomenon was appeared on the tip of the peaks of praziquantel (C7) and sulpiride (C10). It was not significantly improved when chromatographic conditions were optimized. Propranolol hydrochloride (C8) was eluted with a single peak showing no separation of two enantiomers on this kind of chiral column. No peak was detected for atenolol (C9) on the OJ-H column.

The results are shown in Table 2. The chromatograms of C1, C2, C5, C11 and C13 are displayed in Figs. 6–10.

Table 1 Comparisons of samples separation on Chiralcel OD-H.

| Chiral drug          | \( k' \) | \( k_2 \) | Rs  |
|----------------------|---------|---------|-----|
| Nimodipine (C4)      | 5.9     | 6.4     | 1.5 |
| Omeprazole (C6)      | 2.6     | 3.6     | 1.8 |
| Praziquantel (C7)    | 2.3     | 3.0     | 3.4 |
| Propranolol hydrochloride(C8) | 2.7 | 4.4     | 7.6 |
| Atenolol (C9)        | 2.1     | 2.8     | 4.7 |
| Sulpiride (C10)      | 8.9     | 9.9     | 0.9 |

Capacity factor \( k' = (t_1 - t_0)/t_0 \). Resolution (Rs) = \( 2(t_2 - t_1)/(\omega_1 + \omega_2) \). \( t_1 \) and \( t_2 \) are retention times of enantiomer, \( t_0 \) is dead time; \( \omega_1 \) and \( \omega_2 \) are peak widths of enantiomer.

Figure 1 A typical HPLC chromatogram of nimodipine (C4) at conditions of an OD-H column (250 mm × 4.6 mm, i.d., 5 μm) eluting with the mobile phase of diethylamine–n-hexane–isopropanol (0.2:90:10, v/v/v) and detection wavelength set at 254 nm.

Figure 2 A typical HPLC chromatogram of omeprazole (C6) at conditions of an OD-H column (250 mm × 4.6 mm, i.d., 5 μm) eluting with the mobile phase of n-hexane–isopropanol (80:20, v/v) and detection wavelength set at 254 nm.
Cellulose is a linear polymer, which is composed of D(+)-glucose jointed with β-1,4-glycosidic bond or α-1, 4-glycosidic bond. Owing to the chirality on its glucose unit, there is a spiral groove along with the cellulose main chain for each polymer. The enantiomer enters the groove, and then the resolution of enantiomers was implemented through absorption and binding actions.

The mechanism of cellulose chiral stationary phase is complex. Generally speaking, it is a short-term, unstable, diastereomer complex, which is formed between the enantiomer and chiral stationary phase, under the influence of hydrogen bonding, dipole–dipole action, p–p action, electrostatic reaction and complexation. The selectivity and the outward velocity of the enantiomer are determined by the strength of diastereomer bond. The separation is impacted not only by the chiral interaction between groups, also by the

**Figure 3** A typical HPLC chromatogram of praziquantel (C7) at conditions of an OD-H column (250 mm × 4.6 mm, i.d., 5 μm) eluting with the mobile phase of n-hexane-isopropanol (80:20, v/v) and detection wavelength set at 254 nm.

**Figure 4** A typical HPLC chromatogram of propranolol hydrochloride (C8) at conditions of an OD-H column (250 mm × 4.6 mm, i.d., 5 μm) eluting with the mobile phase of n-hexane-isopropanol (80:20, v/v) and detection wavelength set at 254 nm.

**Figure 5** A typical HPLC chromatogram of atenolol (C9) at conditions of an OD-H column (250 mm × 4.6 mm, i.d., 5 μm) eluting with the mobile phase of diethylamine-n-hexane-isopropanol (0.2:70:30, v/v/v) and detection wavelength set at 276 nm.

### 3.1.3. Mechanism investigation of chiral drugs on cellulose chiral column

Cellulose is a linear polymer, which is composed of D(+)-glucose jointed with β-1,4-glycosidic bond or α-1, 4-glycosidic bond. Owing to the chirality on its glucose unit, there is a spiral groove along with the cellulose main chain for each polymer. The enantiomer enters the groove, and then the resolution of enantiomers was implemented through absorption and binding actions.
spatial structure of the stationary phase. The amino and carbonyl on the OJ and OJ-H chiralcel column action point can be in the hydrogen bonding action with the tested molecular. Additionally, the carbonyl can be in the dipole–dipole action with the tested molecular. Moreover, the withdrawing electrons and repelling electrons of the substituent have some impact on the chiral separation.

The chiral recognition of cellulose derivatives CSPs, to a large extent, depends on its highly ordered helical structure. The polar carbamate group and phenyl group reside in the spiral groove of the main chains. There is hydrogen bonding between separated solute molecular and polar carbamate group, C=O, N–H. Dipole–dipole action maybe exits between C=O and C=O. Complex is produced through π–π action between solute molecular and phenyl group. The substituent on the benzene ring takes a great effect on the chiral recognition of CSPs. The hydrogen bonding is in first priority on chiral recognition among all the substituents. All the chiral compounds in this experiment contain benzene ring [9].

Ibuprofen (C1) and ketoprofen (C2) have a similar chemical structure (Fig. 11). Ketoprofen (C2) can be easily separated on Chiralcel OJ-H column, while ibuprofen (C1) has a small resolution over Chiralcel OJ-H column. It has some relation to their structures. The meta position of phenyl ring of ketoprofen (C2) is carbonyl, which has the withdrawing electron action. Also, there is an angle on ketoprofen (C2)’s two aromatics. This angle will be in favor of π–π action between aromatic and stationary phase, also be benefit to form the hydrogen bonding interactions between carbonyl and the stationary phase. The aromatic para site of ibuprofen (C1) is alkyl group, which has the electron-donating effect. It has comparably little contributed to the chiral recognition and barrier the action of ibuprofen (C1) and stationary phase, to some extent, even perform the steric hindrance action.

It can be concluded from the above behavior of chiral separation that the cellulose CSPs are fit for multiple chiral separations, especially for the drugs with aromatics. It also has a good recognition ability to the phenol (such as ibuprofen (C1), ketoprofen (C2)), ester (nitrendipine (C3), nimodipine (C4), felodipine (C5)), ketone (praziquantel (C7)), alcohol (propranolol hydrochloride (C8), atenolol (C9)), and S (omeprazole (C6), sulpiride (C10)).

| Chiral drug         | \( k_1' \) | \( k_2' \) | Rs  |
|---------------------|------------|------------|-----|
| Ibuprofen (C1)      | 1.3        | 1.4        | 1.4 |
| Ketoprofen (C2)     | 2.3        | 3.1        | 6.5 |
| Nitrendipine (C3)   | 2.1        | 2.6        | 0.7 |
| Nimodipine (C4)     | 1.7        | 2.1        | 1.2 |
| Felodipine (C5)     | 2.6        | 3.6        | 1.4 |
| Omeprazole (C6)     | 2.3        | 2.4        | 1.0 |
| Praziquantel (C7)   | 2.3        | 2.4        | 0.3 |
| Sulpiride (C10)     | 4.6        | 5.3        | 0.4 |
| Clenbuterol hydrochloride (C11) | 2.0    | 2.4        | 1.5 |
| Chlorphenamine maleate (C13) | 1.4    | 1.5        | 2.0 |

Table 2  Comparisons of samples separation on Chiralcel OJ-H.

Figure 6  A typical HPLC chromatogram of ibuprofen (C1) at conditions of an OJ-H column (250 mm × 4.6 mm, i.d., 5 μm) eluting with the mobile phase of n-hexane–isopropanol (90:10, v/v) and detection wavelength set at 254 nm.

Figure 7  A typical HPLC chromatogram of ketoprofen (C2) at conditions of an OJ-H column (250 mm × 4.6 mm, i.d., 5 μm) eluting with the mobile phase of n-hexane–isopropanol (80:20, v/v) and detection wavelength set at 254 nm.
3.2. Other impact factor to separation

3.2.1. Impact of diethylamine on separation

According to the results of basic drugs, adding appropriate amount of diethylamine to the mobile phase could inhibit the diastereoselective adsorption of remnant silanol group on the surface of silica gel, contributing to the chiral recognition ability of stationary phase and better separation efficiency, such as propranolol hydrochloride (C8) and atenolol (C9). To acidic drugs, adding diethylamine made separation efficiency worse, taking ibuprofen (C1), ketoprofen (C2) and praziquantel (C7) for example. To neutral drugs, adding diethylamine did not affect the separation efficiency like nimodipine (C3), nitrendipine (C4) and felodipine (C5). The effect of amount of diethylamine was studied simultaneously. The results indicated that the more diethylamine added in the range of 0.1–0.5%, the better the separation efficiency was. However, excessive amount of diethylamine would shorten the life of column. By comprehensive consideration, the compounds reached the
baseline separation as the adding amount was 0.2%, regarded as the appropriate amount.

The effects of organic additives on the racemates separation were investigated. The results are shown in Tables 3 and 4.

3.2.2. Impact of proportion of n-hexane–isopropanol

The impact of proportion of n-hexane-isopropanol was studied to separate the chiral drugs by means of OJ column as the example. Content of isopropanol in the mobile phase would produce a predominant impact on the chiral compounds separation which is shown in Table 5. Results had displayed that the decrease of isopropanol amounts in the mobile phase could lead to the increase of the revolution values. For example, the resolution of clenbuterol hydrochloride (C11) changed from 1.01 to 4.76 when the proportion of isopropanol was adjusted from 20% to 5%. On the other hand, results of separation for some chiral compounds (such as omeprazole (C6) and chlorphenamine maleate (C13)) with bis-phenyl in their structures also implied that the capacity factor (k) of the enantiomer increased when the content of the isopropanol in the mobile phase decreased correspondingly. The possible reason was that decrease of isopropanol led to the polarity of the solvent reduced. It strengthened the hydrogen bonding between the solute and the CSPs and weakened the hydrogen bonding alcohol and CSPs, which produced the increases of the retention time and Rs. Data in Table 5 showed that the increase of n-hexane proportion in favor of separation of two enantiomers.

3.2.3. Impact of column temperature on separation

Column temperature was an important parameter to chiral separation. As column temperature increased, it could accelerate the transfer of solute between the stationary phase and mobile phase and cause diminution of the retention factors. At the same time, the variation of the column temperature would change the equilibrium state of the ions, which might finally impact the chiral acting force between solute and stationary phase.

Experiment of the impact of column temperature on the retention factor (k') and resolution (Rs) was carried out when

| Table 3 | Impact of proportion of diethylamine on separation on Chiralcel OJ-H. |
|-----------------|---------------------------|---------------------------|---------------------------|---------------------------|
| **Chiral drug** | **Rs(no organic additives)** | **Rs(0.1% diethylamine)** | **Rs(0.2% diethylamine)** | **Rs(0.5% diethylamine)** |
| Ibuprofen (C1) | 1.4 | – | – | – |
| Ketoprofen (C2) | 6.5 | – | – | – |
| Nitrendipine (C3) | 0.7 | 0.6 | 0.7 | 0.7 |
| Nimodipine (C4) | 1.2 | 1.1 | 1.2 | 1.1 |
| Felodipine (C5) | 1.4 | 1.5 | 1.4 | 1.4 |
| Omeprazole (C6) | 1.0 | 1.0 | 1.2 | 1.1 |
| Praziquantel (C7) | 0.25 | – | – | – |
| Sulpiride (C10) | 0.36 | 2.1 | 3.1 | 3.4 |
| Clenbuterol Hydrochloride (C11) | 0.23 | 1.3 | 2.2 | 2.6 |
| Chlorphenamine maleate (C13) | – | 1.2 | 2.0 | 2.3 |

Note: “–” means not separated.

| Table 4 | Impact of proportion of diethylamine on separation on Chiralcel OD-H. |
|-----------------|---------------------------|---------------------------|---------------------------|---------------------------|
| **Chiral drug** | **Rs(no organic additives)** | **Rs(0.1% diethylamine)** | **Rs(0.2% diethylamine)** | **Rs(0.5% diethylamine)** |
| Nimodipine (C4) | 1.3 | 1.4 | 1.5 | 1.4 |
| Omeprazole (C6) | 1.8 | 2.0 | 2.0 | 2.1 |
| Praziquantel (C7) | 3.4 | 3.5 | 3.4 | 3.4 |
| Propranolol hydrochloride (C8) | 4.6 | 6.1 | 8.7 | 12.2 |
| Atenolol (C9) | 3.9 | 4.0 | 4.7 | 4.5 |
| Sulpiride (C10) | 0.9 | 0.9 | 0.7 | 0.8 |

| Table 5 | Impact of proportion of n–hexane-isopropanol on the chiral separation. |
|-----------------|---------------------------|---------------------------|---------------------------|---------------------------|
| **Proportion of n-hexane–isopropanol (v/v)** | **Resolution (Rs)** |
| | Ibuprofen (C1) | Omeprazole (C2) | Clenbuterol hydrochloride (C11) | Chlorphenamine maleate (C13) |
| 80:20 | 0.74 | 1.01 | 1.01 | Undivided |
| 90:10 | 1.22 | 1.42 | 2.48 | 1.14 |
| 95:5 | 1.37 | 1.83 | 4.76 | 1.95 |
| 98:2 | 1.45 | 1.96 | 5.31 | 2.04 |
n-hexane–isopropanol (90:10) was adopted. Within the range of certain temperature, the retention factor ($k^0$)s of all the enantiomers kept a decrease trend towards the temperature increased, but the degree of decrease was different. The results indicated that the separation of most racemates got the maximum value with increasing temperature, and increased in the range of 15–20°C, kept constant at 25°C, intended to decrease at 30–40°C, where as the peak shape of nitrendipine (C3), nimodipine (C4) and felodipine (C5) was optimized on OJ column and thus the separation was improved.

It could be concluded that the temperature has some influence on the separation and the peak shape. In order to achieve the stable separation result, the effect of column temperature should be studied in the process of analysis so as to obtain the optimum temperature and keep it unchanged. The results are shown in Tables 6 and 7.

4. Conclusion

In this paper, it was first time to separate the thirteen chiral drugs recorded in Ch.P2010 by two cellulose ramification chiral stationary phase, chiralcel OD and chiralcel OJ, and separation mechanism was also studied by comparison of these two columns. Thirteen types of racemates from Ch.P2010 have been selected which are commonly applied in clinics such as β-receptor blocking drugs, benzamide antipsychotic drugs, calcium antagonist, antihistamine drugs, proton pump inhibitor, dihydropyridine calcium channel blockers, antipyretic analgesics, and broad-spectrum antiparasitic agents. The chemical structures of these target racemates included amines, alcohols, ethers, ketones, acylamide, halogenated aromatic hydrocarbons, polycyclic compounds, and heterocyclic compounds. The result displayed that the cellulose chiral stationary phase could be widely used to separate many compounds in normal phase HPLC.

To obtain the optimal resolution, chromatographic conditions were studied by means of adjusting several parameters including concentrations of organic additives and column temperature. The chromatographic retention and resolution of the enantiomers can be adjusted through the change of the alcohol displacer content of the mobile phase, the concentration of organic alkali modifiers and the column temperature. The study could provide methodological basis for the enantiomers separation to study and control the optical impurity in the future work.

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Table 6  Impact of column temperature on separation on Chiralcel OJ-H.

| Chiral drug        | Rs (18°C) | Rs (25°C) | Rs (35°C) |
|--------------------|-----------|-----------|-----------|
| Ibuprofen (C1)     | 1.1       | 1.4       | 1.2       |
| Ketoprofen (C2)    | 4.3       | 6.5       | 3.8       |
| Nitrendipine (C3)  | 0.6       | 0.7       | 1.0       |
| Nimodipine (C4)    | 1.0       | 1.2       | 1.6       |
| Felodipine (C5)    | 0.8       | 1.4       | 1.9       |
| Sulpiride (C10)    | 2.8       | 3.1       | 3.1       |
| Clenbuterol        | 1.5       | 2.2       | 1.9       |
| Hydrochloride (C11)| 1.7       | 2.0       | 1.8       |

Table 7  Impact of column temperature on separation on Chiralcel OD-H.

| Chiral drug       | Rs (18°C) | Rs (25°C) | Rs (35°C) |
|-------------------|-----------|-----------|-----------|
| Nimodipine (C4)   | 1.4       | 1.5       | 1.5       |
| Omeprazole (C6)   | 1.8       | 2.0       | 1.9       |
| Praziquantel (C7) | 3.4       | 3.4       | 3.1       |
| Propranolol        | 8.1       | 8.7       | 8.4       |
| Hydrochloride (C8) |          |           |           |