Received: 9 April 2019 | Revised: 17 July 2019 | Accepted: 18 July 2019

DOI: 10.1002/chir.23118

REGULAR ARTICLE

WILEY

# HPLC and SFC enantioseparation of (±)-Corey lactone diol: Impact of the amylose tris-(3,5-dimethylphenylcarbamate) coating amount on chiral preparation

**Huiying Wang | Qiuhua Wang | Yaling Wu | Lingping Cheng | Lunan Zhu | Junchen Zhu | Yanxiong Ke**

Engineering Research Center of Pharmaceutical Process Chemistry, School of Pharmacy, Ministry of Education, East China University of Science and Technology, Shanghai, China

**Correspondence**
Yanxiong Ke, Engineering Research Center of Pharmaceutical Process Chemistry, School of Pharmacy, Ministry of Education, East China University of Science and Technology, Meilong Road 130, Shanghai, China.
Email: key@ecust.edu.cn

**Funding information**
National Natural Science Foundation of China, Grant/Award Number: 21375038

**Abstract**

As an important intermediate of prostaglandins and entecavir, optically pure Corey lactone diol (CLD) has great value in the pharmaceutical industry. In this work, the enantioseparation of (±)-CLD was evaluated using high-performance liquid (HPLC) and supercritical fluid chromatography (SFC). In HPLC, the separations of CLD enantiomers on polysaccharide-based chiral stationary phases with both normal phase and polar organic phase were screened. And the conditions for the enantioseparation were optimized in HPLC and SFC, including the selection of mobile phase, temperature, back-pressure, and other conditions. More important, it was found that the chiral resolutions were greatly enhanced by the increase of the coating amount of ADMPC (amylose tris-(3,5-dimethylphenylcarbamate)) under both HPLC and SFC conditions, which can lead to the increase of the productivity and the decrease of the solvent consumption. The preparations of optically pure CLD were evaluated on a semi-preparative (2 × 25 cm) column packed with 30% ADMPC-coated CSP under HPLC and SFC conditions. Preparative performances in terms of kkd are 1.536 kg racemate/kg CSP/day and 1.248 kg racemate/kg CSP/day in HPLC and SFC, respectively.

**KEYWORDS**

chiral separation, chiral stationary phase, mobile phase, polar organic mobile phase, semi-preparation

## 1 | INTRODUCTION

Prostaglandins (PGs) are a class of endogenous natural products with biological activity. PGs have the advantages of mild toxicity to the human body and have various physiological regulatory functions on the human body. Therefore, PGs are widely used in the field of medicine. PGs derivatives have succeeded in becoming an important class of drugs, offer advantages in the treatment of idiopathic pulmonary hypertension,[1] termination of early pregnancy,[2] immune and inflammatory response,[3] human colon cancer cells,[4,5] and gastrointestinal tract illnesses.[6]

In 1969, Corey first proposed that the use of Corey lactone diol (CLD) in the synthesis of prostaglandin could control its stereochemistry at an early stage, and all the primary prostaglandins and a variety of analogs could be synthesized from this precursor.[7] The synthesis of PGs has been greatly simplified by using (−)-CLD. Nowadays (−)-CLD has become the key intermediate in the syntheses of various types of prostanoidins industry. In

  

addition, (+)-CLD is also an intermediate in the synthesis of entecavir, a medicine for anti-virus therapy of chronic hepatitis B. According to the statistics by World Health Organization, more than 2 billion of the world's residents are infected with hepatitis B virus (HBV).[8] Entecavir is the most selective against HBV. Long-term renal tolerance of entecavir was good and side effects were minimal. An extensive review on this topic including patent data has been published by Slova.[9] Recent reports showed that the synthetic steps of entecavir could be largely shortened from the CLD.[10,11] The structure of CLD is shown in Figure 1.

Up to the date, many efforts have been done to prepare optically pure CLD because of its great value in the pharmaceutical industry. Records included asymmetric synthesis,[12] chiral pool synthesis,[13] and chiral separation.[14,15] The asymmetric synthesis and chiral pool synthesis have been developed to synthesize optically pure CLD.[14,16] However, these methods often suffered low overall yields because of their requirements of multiple reaction steps in the syntheses.[17] Crystallization of CLD derivatives and enzymatic kinetic resolution methods were reported to separate CLD enantiomers.[18] In recent decades, direct chiral separation using chiral stationary phases (CSPs) in high-performance liquid chromatography (HPLC) has become a popular and reliable tool for enantioseparation. It has been reported that CLD enantiomers could be separated on commercial 20 μm Chiralpak AD and Chiral AS chiral stationary phases (CSPs).[19]

Polysaccharide derivatives are the most widely used CSPs in the analysis of enantiomer compositions and the preparation of pure enantiomers. It has been reported that the highly ordered helical structure enhanced the enantiorecognition abilities of the polysaccharide derivatives.[20] The enantioselectivities of polysaccharide-based CSPs not only depend on the structures of polysaccharide derivatives but also on the coating method and coating amount of the chiral selectors. For example, there are reports that investigated the separation of chrysanthemate isomers on the CSP of cellulose tris-(4-methylbenzoate) (CTMB), and the separation factor for chrysanthemic acid ethyl ester significantly depended on the preparation conditions of the CSP, such as the coating amount of CTMB and the type and amount of coating solvents.[21] Another literature has shown that the amount of cellulose tris-(3, 5-dimethylphenylcarbamate) (CDMPC) adsorbed on the silica gel greatly influenced the chiral recognition of some racemates. Loading capacity of racemates increased with an increase of the amount of CDMPC supported on the silica gel.[22] These results indicate that the optimization of coating method and coating amount of polysaccharide-based CSP are important for chiral separation in HPLC, especially for the method development with the purpose of chiral preparation, because small increases in selectivity, resolution and loading capacity will yield major increases in productivity in large-scale separations.[23]

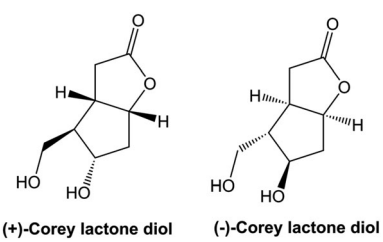


**FIGURE 1** The structure of Corey lactone diol

Besides the chiral selectivity and resolution, the sample loading capacity is another critical factor that influences the separation efficiency in chiral preparative chromatography, which is closely related to solubility of solute in the mobile phase. (±)-CLD is highly soluble in water, well soluble in alcohol and sparely soluble in alkane. Compared with traditional normal phase liquid chromatography (NPLC), polar organic solvent chromatography (POSC) is more preferred for the preparative purpose because (±)-CLD has a much better solubility in the solution. In recent years, supercritical fluid chromatography (SFC) is more and more popular in enantioseparation because of its advantages, such as high efficiency, less organic solvent consumption, and easy to workup. Moreover, the characteristics of the low viscosity of supercritical $CO_2$ and the high mass transfer allow SFC to use of a more efficient media, such as a small particle chiral stationary phase (5 μm), with a much faster flow rate in the separation, which can lead to the significant improvement of the separation efficiency.

The aim of this work was to develop a more efficient chromatographic separation method for the preparation of optically pure CLD. Both HPLC and SFC were explored when undertaking the method development for (±)-CLD enantioseparation. The type of polysaccharide-based CSP, mobile phase, mobile phase additive, and temperature were optimized. More importantly, the impact of the coating amount of amylose tris-(3,5-Dimethylphenylcarbamate) (ADMPC) on the separation efficiency was also investigated. The CSP with a high coating amount of ADMPC gave a much higher selectivity and resolution for CLD enantiomers. The chiral preparations of (±)-CLD were performed on a semi-preparative column (250 × 20 mm i.d. 5 μm) under HPLC and SFC conditions.

## 2 | MATERIALS AND METHODS

### 2.1 | Chemicals

(±)-CLD and optically pure CLD were supplied by Bide Pharmatech Ltd (Shanghai, China). Four packed columns

Chiralpak AD-H, Chiralcel OD-H, Chiralcel OJ-H, and Chiralpak AS-H (250 × 4.6 mm i.d.) were obtained from Daicel Chiral Technologies (Shanghai, China) Co., Ltd. Spherical silica gel (5 μm, 1000 Å, 30 $m^2$/g) was provided by Acchrom Ltd (Beijing, China). Other important chemicals of this research are listed as below: DMF and THF were purchased from Titan Technologies Inc (Shanghai, China). All solvents and reagents used were of HPLC grade: methanol, ethanol, ACN (acetonitrile), and n-hexane obtained from J&K Chemicals (Shanghai, China).

## 2.2 | Packing materials

ADMPC was synthesized according to the reported method.[24] Amylose of 15 g was reacted with 52.5 g of 3,5-dimethylphenyl isocyanate in 105 mL anhydrous pyridine at 100°C for 20 hours. The reaction solution was poured into 1 L methanol, and the precipitate was collected by filtration and washed with large amount of methanol and dried at 50°C under vacuum for 12 hours to yield 54 g of ADMPC.

The elemental analysis data found w (N) %, w (C) %, and w (H) % were very closed to the calculated data, which showed that all of the hydroxyl groups in amylose were almost converted to carbamate groups (Anal. Calcd. for ADMPC: C, 65.66; H, 6.18; N, 6.96; found: C, 64.65; H, 6.09; N, 7.01).

FTIR ($cm^{-1}$) 3382, 3320 ($\nu_{NH}$), 1722 ($\nu_{C=O}$), which also indicates that the hydroxyl groups of the amylose have been converted into the carbamate moieties.

The prepared ADMPC was coated on 5 μm silica gel. The ratio of the derivative to the silica gel (*w*/w) was 1:10; 2:10; 3:10, and 4:10, respectively, denoted as ADMPC-10, ADMPC-20, ADMPC-30, and ADMPC-40, respectively.

The details of preparation and characterization of ADMPC and the packing materials were described in the Supporting Information.

## 2.3 | Column packing

A slurry packing procedure was employed to pack the chromatography columns (250 × 4.6 mm i.d. for HPLC, 150 × 4.6 mm i.d. for SFC, and 250 × 20 mm i.d. for semi-preparation). Briefly, the stationary phase was suspended in methanol and ultrasonicated for 15 minutes. Methanol was employed as packing solvent. The column was packed at 400 to 500 kg/$m^2$ on an SP-100 liquid chromatography column packing machine supplied by Dalian Sipore Co (Dalian, China) with a DSF-100 air-driven hydraulic pump provided by Mpapower (Shanghai, China).

## 2.4 | Instrumentation and chromatographic conditions

The morphologies and microstructure of ADMPC were observed by a Hitachi S-3400 N scanning electron microscope (SEM) with 15 kV accelerating voltage. And the FT-IR spectra of ADMPC were recorded on a NICOLET 6700 FT-IR spectrophotometer.

Analyses in NPLC mode were carried out by HPLC system equipped with a 515 pump (Waters, Milford, Massachusetts), a 7725i manual injector and a 2489 U*V*/*v*isible detector (Waters, Milford, Massachusetts). Chromatographic analyses in POSC mode were performed on an Alliance HPLC system (Waters, Milford, Massachusetts) consisted of a 2695 pump and a 2998 UV/visible detector. SFC separations were performed on the system of Waters ACQUITY Ultra Performance Convergence Chromatography (ACQUITY UPC2), which consists of a binary solvent delivery pump, as sampler manager-FL, a column manager Aux, a photodiode array (PDA) detector, and an automatic backpressure regulator (ABPR). All data acquisition and processing were conducted by the Waters Empower Pro 3 software.

The preparative POSC was carried out on a Waters Auto purification System, which includes a 2545 binary gradient module, a 2767 sample manager, and a 2489 U*V*/*v*isible detector. Data were collected using a Masslynx4.1 workstation.

The preparative SFC was performed using an SFC prep-80 system, which includes a high-pressure $CO_2$ pump, a high-pressure co solvent pump, a mass flow meter, a PDA detector, an ABPR, a manual backpressure regular, and six high-pressure fraction collection cyclones (Waters, Milford, Massachusetts). Data acquisition and processing were conducted by SuperChrom software.

(±)-CLD sample was dissolved at the concentration of 10 mg/mL in mobile phase for HPLC analysis and 10 mg/mL in methanol for SFC analysis, respectively. The injection volume was 5 μL for analyses. Column temperature was controlled at 20°C in HPLC and 35°C in SFC if there were no other specifications. Detection wavelength was set at 220 nm.

1,3,5-tri-*tert*-butylbenzene (TTB) is generally used as the non-retained compound for estimating the dead time ($t_0$) under the same condition as column efficiency determination.[25] The dead times of the columns were measured by using 1, 3, 5-tri-tert-butylbenzene (TTB) with the mobile phase of hexane/isopropanol (90/10, v/v). The measured dead times on ADMPC-10, ADMPC-20, ADMPC-30, and ADMPC-40 were 3.07, 2.75, 2.37, and 2.03, respectively. These values were used for the calculation of the selectivity of CLD enantiomers in HPLC. The dead time of the SFC was determined by the retention time of the solvent peak.

## 3 | RESULTS AND DISCUSSION

### 3.1 | HPLC separation of (±)-CLD

#### 3.1.1 | Choice of chiral column

The various of polysaccharide-based chiral analytical HPLC columns (Chiralpak AD-H, Chiralcel OD-H, Chiralcel OJ-H, and Chiralpak AS-H) and mobile phases (a normal mobile phase consisted of n-hexane/ethanol (80/20, v/v) and a polar organic mobile phase consisted of 100% ACN) were screened. The separation results are shown in Table 1. The elution order was determined by the injection of optically pure CLD standard sample.

No chiral resolution was observed on the Chiralcel OD-H and Chiralcel OJ-H columns with either normal mobile phase or polar organic mobile phase. The best resolution was achieved on the Chiralpak AD-H column with separation factor ($\alpha$ value) of 2.03 and resolution factor ($R_S$ value) of 5.17 in ACN. Normal phase also gave satisfied separation result on this column with $\alpha$ value of 1.53 and $R_S$ value of 4.75. Under normal phase condition, CLD enantiomers were also separated on the Chiralpak AS-H column with a lower separation factor and a lower resolution factor. Only a limited resolution was achieved on this column with a mobile phase of 100% ACN. It should be noted that the elution order of the two enantiomers on the Chiralpak AD-H was different from that on the Chiralpak AS-H column. (+)-CLD was eluted first on the Chiralpak AD-H column, while (−)-CLD was eluted first on the Chiralpak AS-H column.

For preparative chromatography, the sample solubility is a crucial issue. In this case, the satisfied separation was obtained with the normal mobile phase on the Chiralpak AD-H column. However, the solubility of (±)-CLD was very low in this solvent, which was impractical for preparative purposes. A high selectivity under high-solubility conditions would be more favorable for preparative separation. Thus, the enantioseparation of (±)-CLD on the Chiralpak AD-H with polar organic mobile phase was carried out in the following optimization.

**TABLE 1** The separations of Corey lactone diol (CLD) on the chiral stationary phases (CSPs)

| Column | $k_1$ | $\alpha$ | $R_s$ | EO | $N_1$ | $M_P$ | Solubility (mg/mL) |
|---|---|---|---|---|---|---|---|
| AD-H | 7.75 | 1.53 | 4.75 | (+) | 2190 | A | |
| | 0.65 | 2.03 | 5.17 | (+) | 6558 | B | ~55 |
| | 0.62 | 2.35 | 4.95 | (+) | 1500 | C | ~110 |
| | 0.67 | 2.17 | 4.94 | (+) | 1690 | D | ~100 |
| | 0.38 | 1.96 | 3.76 | (+) | 5535 | E | ~200 |
| AS-H | 1.74 | 1.47 | 2.71 | (−) | 2422 | A | |
| | 0.36 | 1.37 | 1.03 | (−) | 2729 | B | ~55 |

*Note*. EO, elution order, absolute configuration of the first-eluted enantiomer. $N_1$, column efficiency (no. of theoretical plates, /m) of first-eluted enantiomer. $M_P$, mobile phase, A, hexane/ethanol (80/20, v/v); B, ACN; C, ACN/methanol (97:3, v/v); D, ACN/ethanol (97:3, v/v), E, ACN/water (97:3, v/v); flow-rate: 1.0 mL/min.

#### 3.1.2 | Influence of polar modifiers in polar organic mobile phase

ACN is an attractive mobile phase for preparative chromatography because of its low viscosity. The polar modifiers of methanol, ethanol, and $H_2O$ were evaluated using ACN/modifier (97:3, v/v) mobile phases. The solubility of (±)-CLD of in these mobile phases were measured. The separation results and the solubility values were summarized and listed in Table 1.

The sample solubility was estimated by weighting 100 mg of sample into the vials. A certain amount of selected solvents were added into the vials and stirred at a temperature of 25°C. The solubility of (±)-CLD was only about 55 mg in ACN. It was dramatically increased to about 200 mg/mL in ACN/water (97:3, v/v). The solubility in ACN/methanol (97:3, v/v) and ACN/ethanol (97:3, v/v) solution was comparable, which was about the half of that in ACN/$H_2O$ (97:3, v/v) solution.

The retentions of both enantiomers decreased with the increase of modifier's polarity. The selectivity and the resolution were 1.96 and 3.76, respectively, with water as co-solvent, which were lower than those with the co-solvent of methanol or ethanol. However, the column efficiency was much better when using water as a modifier. Also, considering the good resolution, the high solubility in this mobile phase and the convenience of the solvent recovery, water was used in the preparation application.

The influence of water concentration on the separation was examined. As shown in Figure 2, the influence of water concentration on the selectivity was small. Both of the retention and resolution kept increasing with the decrease of water concentration. Thus, concentration of water in mobile phase should be as small as possible when the solubility of (±)-CLD was acceptable for preparative purpose.

#### 3.1.3 | Influence of temperature

The column temperature is expected to influence the enantioselectivity in chiral separation procedures.[26] The retention factor and separation factor will associate with the column temperature.[27] In order to elucidate the thermodynamic characteristics of the chiral recognition process as well as to optimize the separation selectivity, the

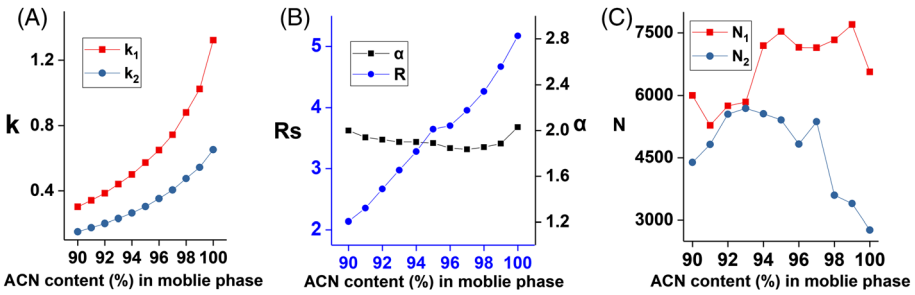


**FIGURE 2** Plots of the retention (A), separation factor and resolution factor (B), and column efficiency (C) of (±)-CLD in the different composition of mobile phases (acetonitrile-water). Chromatographic conditions: Column, Chiralpak AD-H; mobile phase, acetonitrile-water mixtures; flow-rate, 1.0 mL/min

enantioseparation data were recorded on the Chiralpak AD-H column with stepwise raise over the range of 20°C to 55°C in 5°C increments. The obtained chromatographic data were correlated to the adsorption thermodynamic parameters by the following Van't Hoff equation:

$$\ln k = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} + \ln\Phi \quad (1)$$

$$\ln\alpha = -\frac{\Delta\Delta H}{RT} + \frac{\Delta\Delta S}{R} \quad (2)$$

$$G = H - TS, \quad (3)$$

where $k$ represents the retention factor; $R$ is the universal gas constant (8.3144 J mol$^{-1}$ K$^{-1}$); $T$ is the absolute temperature; $\Delta H$ and $\Delta S$ are the molar enthalpy and molar entropy of the adsorption; $\Phi$ is the column phase ratio, $\Delta\Delta H$, $\Delta\Delta S$, and $\Delta\Delta G$ are the differences of the molar differential enthalpy, entropy, and Gibbs-free energy of the enantioselective adsorption, respectively.

As shown in Figure 3, the fitted curve of lnk versus $1/T \times 10^{-3}$ and $\ln\alpha$ versus $1/T \times 10^{-3}$ possessed high values of regression coefficients (linear correlation coefficient $R^2 > .99$). The linear plots indicated that these thermodynamic parameters were constant, which meant that the enantioselective mechanism remained unchanged. $\Delta H_0$ was less than 0, indicating that the adsorption of CLD enantiomer on the stationary phase was an exothermic process. The negative value of $\Delta\Delta H$ ($-3.6 \pm 0.1$ kJ mol$^{-1}$) indicated that the enantioseparation of CLD was predominantly enthalpically driven. A negative $\Delta\Delta S$ value ($-8.1 \pm 0.2$ J mol$^{-1}$ K$^{-1}$) indicates that (−)-CLD had less degree of freedom in binding to the chiral selector compared with (+)-CLD. Thus, a lower temperature would be preferred for the separation of CLD enantiomers since the term of $-T\Delta\Delta S$ will cause the increase of $\Delta\Delta G$ in Equation (3) at an elevated temperature. As expected, the separation factor increased from 1.44 to 1.70 by lowering the temperature from 55°C to 20°C. The resolution value also increased by lowering the temperature from $R_s = 1.46$ at 55°C to $R_s = 3.76$ at 20°C.

### 3.1.4 | Impact of ADMPC coating amount on HPLC enantioseparation

The improvement of selectivity and resolution can lead to a higher sample loadability, and thus increase the productivity and decrease the consumption of solvent. Up to the day, most of the investigations mainly focus on the CSP selection and the mobile phase optimization. It has been

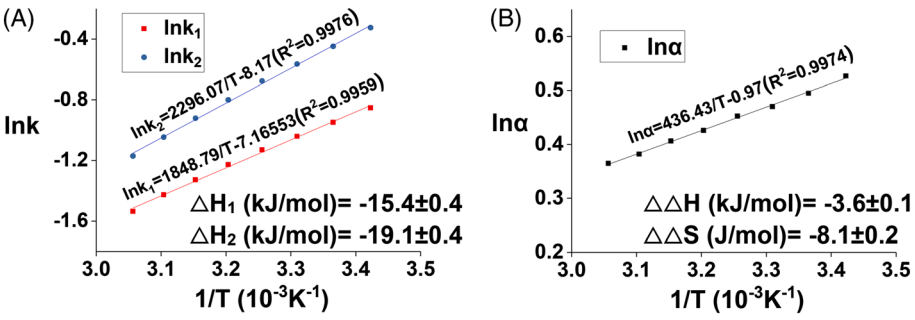


**FIGURE 3** Retention (A) and separation (B) factors versus $1/T$ of chiral separation of Corey lactone diol (CLD). Chromatographic conditions: Column, Chiralpak AD-H; mobile phase, ACN/water (97:3, v/v); flow-rate, 1.0 mL/min

reported that the selectivity of polysaccharide-based CSP is also affected by the coating amount of the polysaccharide derivative. The enantiorecognition ability of polysaccharide-based CSPs not only depends on the type of chiral selector, but also on its spatial configuration. A higher amount coating of polysaccharide derivative on the stationary phase may result in a more regular conformation and orientation, which can lead to more chiral recognition sites and enhanced the chiral selectivity and resolution for some chiral compounds.

Here, four chromatographic columns (ADMPC-10, ADMPC-20, ADMPC-30, and ADMPC-40) with the increasing of ADMPC coating amount were prepared, and their performances were evaluated. The column was first tested using trans-stilbene oxide (TSO) as a standard with the mobile phase of hexane/isopropanol (90/10, v/v). As shown in Figure 4, the retention and selectivity of TSO enantiomers kept increasing with the increase of the ADMPC coating amount. It was interesting to find that the separation factors were even better than those of the

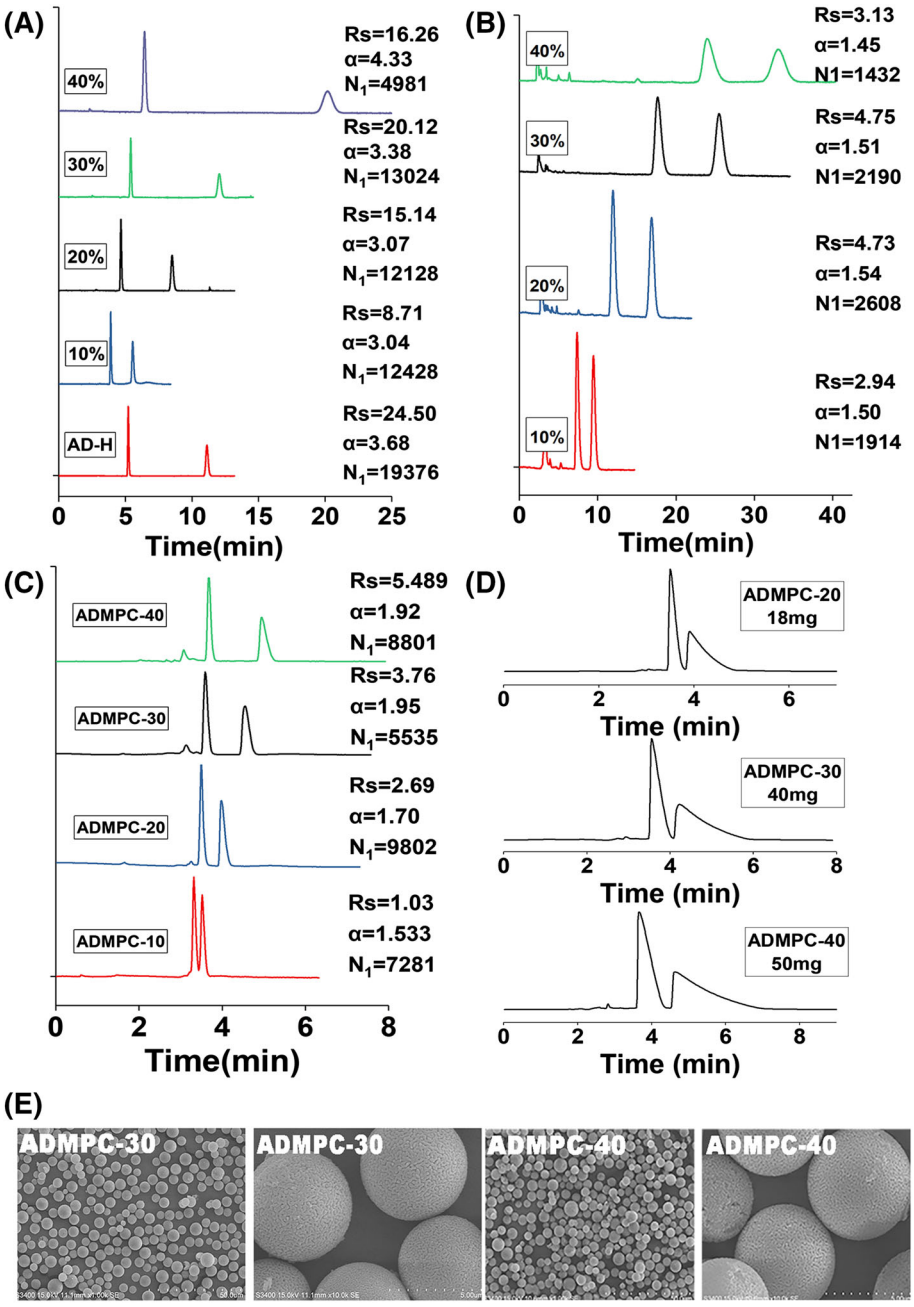


**FIGURE 4** Effect of the coating amount of ADMPC on the separation of (A) TSO in n-hexane/isopropanol (90/10, v/v), (B) (±)-CLD in n-hexane/ethanol (80/20, v/v) and (C) (±)-CLD in ACN/water (97/3, v/v). Flow-rate: 1.0 mL/min; injection: 2 μL, 4 mg/mL. (D) The effect of ADMPC coating amount on maximum loading capacity. Chromatographic conditions: column: ADMPC-20, ADMPC-30, ADMPC-40; mobile phase: ACN/water (97/3, v/v); flow-rate: 1.0 mL/min; injection: 40 μL, 200 mg/mL. (E) The SEM images of the ADMPC-30 and ADMPC-40

commercial AD-H column when the coating amount was higher than 30%. Although the selectivity on ADMPC-40 was the highest, the resolution factor was lower than that on ADMPC-30 because of the lower column efficiency. From the SEM images of the ADMPC-30 and ADMPC-40, no obvious particle aggregation was found in Figure 4 E. Both of them were well-coated. The attempt to improve column efficiency of ADMPC-40 by optimizing the column packing condition in our lab failed. We suspected that the high solid-liquid mass transfer resistance caused by the overcoating of ADMPC-40 on the packing material should be responsible for the low column efficiency.

For the separation of CLD enantiomers, the retention and resolution of CLD enantiomers also kept increasing with the increase of ADMPC coating amount. The best separation factor (1.95) was achieved on ADMPC-30, and the best resolution factor (5.49) was obtained on ADMPC-40. It was interesting to find that the column efficiency of ADMPC-40 was comparable with the other three columns. This finding may indicate that the mass transfer mechanism in polar organic phase was different from that in normal phase.

The maximum of sample loading capacities on the columns were evaluated on analytical columns. The loading quantity was constantly increased until enantiomers of CLD on the column could not reach the baseline separation level. As shown in Figure 4D, a higher ADMPC coating amount led to a higher sample loading capacity. The maximum loading capacity dramatically increased from 3.6 mg on ADMPC-20 to 8 mg on ADMPC-30. Further increasing the ADMPC coating amount to 40% increased the sample loading capacity to 10 mg.

We performed stable enantioseparation of (±)-CLD using stacking injection on ADMPC-30 and ADMPC-40, respectively. Each injection interval (the time span from the start of the first peak to the end of second peak) on ADMPC-30 and ADMPC-40 were set at 3 and 4 minutes, respectively. Thus, the estimated injection amount of (±)-CLD sample on ADMPC-30 and ADMPC-40 per hour were 160 and 150 mg, respectively. From this point of view, the throughput on ADMPC-30 was slightly higher than that on ADMPC-40 when taking the separation time into account.

## 3.2 | SFC enantioseparation of (±)-CLD

### 3.2.1 | Optimization of SFC conditions

Under the SFC mode, three common organic modifiers, including methanol, ethanol, and isopropanol, were evaluated on an ADMPC-30 column based on the above results. And the effects of modifier concentration, temperature, and back pressure on the enantioseparation were also investigated.

As shown in Table 2, the CLD enantiomers were not baseline separated when using ethanol as modifier. The using of 20% methanol co-solvent offered much better selectivity ($\alpha = 1.44$) and resolution ($R_S = 3.95$) in SFC. The peak tailing was minimized and the separation was finished in 3 minutes.

With the decrease of methanol concentration, the elution strength of the mobile phase decreased, resulting in an increase of the retention time. The retention factor of the first elution peak dramatically increased from 3.19 to 7.17 when the concentration of methanol decreased from 20% to 10%. And the resolution increased when the percentage of methanol decreased.

In SFC, the increase of the back pressure will increase the density of $CO_2$ supercritical fluid, which will lead to the change of retention. In this experiment, the back pressure had little effect on separation. Only minor changes on retention, selectivity, and resolution were observed when the back pressure was varied from 1.6 kPa to 2.2 kPa.

As shown in Table 2, a slight increase of $k_1$ and a slight decrease of $\alpha$ value were observed with the increase of temperature from 293 to 328 K. The fluctuation of the $R_s$ value was kept in a small range. Normally, the density of $CO_2$ supercritical fluid at a high temperature is lower than that at a lower temperature, which can cause the decrease of elution strength. However, the mass transfer will increase with the increase of temperature. Here, the impact of column temperature on the SFC separation retention was small.

In general, the SFC separation of CLD enantiomers is largely influenced by the type and concentration of modifier. The influences of back pressure and temperature on the enantioseparation are small. These characteristics

**TABLE 2** The effect of different modifiers, temperature, and back-pressure on SFC chiral separation on ADMPC-30

| $M_P$ | $BP$/psi | $T$/K | $k_1$ | $\alpha$ | $R_S$ |
|---|---|---|---|---|---|
| 10% methanol | 2000 | 308 | 7.17 | 1.45 | 4.82 |
| 15% methanol | 2000 | 308 | 5.57 | 1.44 | 4.38 |
| 20% methanol | 2000 | 308 | 3.19 | 1.44 | 3.95 |
| 20% ethanol | 2000 | 308 | 3.87 | 1.10 | 1.11 |
| 20% isopropanol | 2000 | 308 | 2.76 | 1.22 | 2.08 |
| 20% methanol | 1600 | 308 | 3.40 | 1.44 | 3.99 |
| 20% methanol | 1800 | 308 | 3.29 | 1.44 | 4.02 |
| 20% methanol | 2200 | 308 | 3.09 | 1.44 | 3.91 |
| 20% methanol | 2000 | 298 | 3.22 | 1.44 | 3.88 |
| 20% methanol | 2000 | 318 | 3.13 | 1.43 | 4.06 |
| 20% methanol | 2000 | 328 | 3.08 | 1.42 | 3.92 |

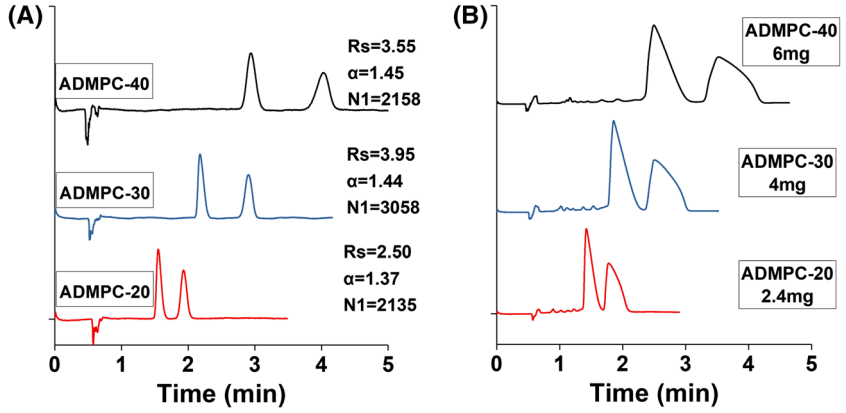


**FIGURE 5** The effect of ADMPC coating amount on the SFC enantioseparation (A) and maximum loading capacity (B). Chromatographic conditions: Column: ADMPC-20, ADMPC-30, ADMPC-40; mobile phase: 10% methanol in $CO_2$; flow-rate: 3.0 mL/min; column temperatures: 308 K; back-pressure: 2000 psi

will make the enantioseparation of (±)-CLD in SFC easy to control.

### 3.2.2 | Impact of ADMPC coating amount on SFC separation

The impact of ADMPC coating amount on the chiral separation was examined in SFC. As expected, the retention and selectivity of the CLD enantiomers increased with increasing coating amount of ADMPC. ADMPC-30 gave the best resolution for (±)-CLD.

The maximum of sample loading capacities on analytical column under SFC condition were tested on ACQUITY UPC$^2$. A CLD solution in methanol with concentration of 600 mg/mL was used for the testing. As shown in Figure 5, the maximum loading of ADMCP-20 and ADMCP-30 were 2.4 mg and 4.0 mg, respectively. Because of the limitation of sample loop equipped on this instrument (the volume of sample loop is only 10 μL), 6.0 mg of the sample was injected in ADMPC-40. From the chromatogram, there was still room for improvement in the sample load. Again, these results showed that the sample loading capacity was improved by the ADMPC coating amount.

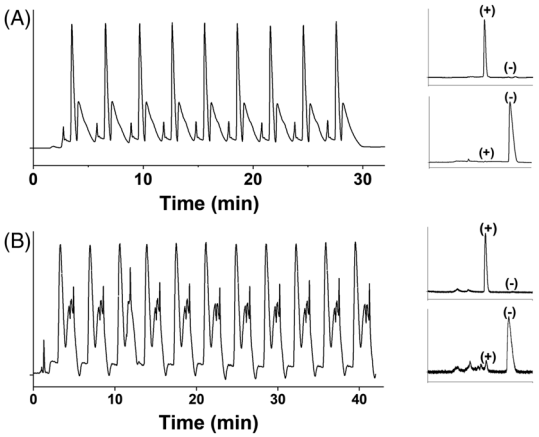


**FIGURE 6** (A) The semi-preparation on ADMPC-30 and the analytical purity of the prepared enantiomers; mobile phase, ACN and water (97:3, v/v); flow-rate, 20.0 mL/min; injection, 160 mg. (B) the semi-preparation on SFC and the analytical purity of the prepared enantiomers; mobile phase, 20% MeOH; flow-rate, 60 g/min; injection, 130 mg

## 3.3 | Semi-preparation

According to the above experimental results, the semi-preparations of CLD enantiomers were performed on a 250 × 20 mm column packed with ADMPC-30 packing material in both HPLC and SFC (Figure 6).

A "stack injection" was used to increase the productivity. The injection cycle time was set at 3 minutes in HPLC and SFC. To separate 1 g CLD, the HPLC method took about 20 minutes with 390 mL ACN consumption, and the SFC method took about 23 minutes with 460 mL methanol and 920 g $CO_2$ consumption. The enantiomeric purities of the two peaks are 98.33% and 99.41% in HPLC, and 99.02% and 94.26% in SFC. Preparative performances in terms of kkd (kilogram of compound purified per kilogram of stationary phase per day) are 1.536 kg racemate/kg CSP/day and 1.248 kg racemate/kg CSP/day in HPLC and SFC, respectively. On the basis of the above results, the HPLC method can provide higher productivity and less solvent consumption. Thus, the HPLC method is a better choice for CLD enantio-preparation.

## 4 | CONCLUSION

In this paper, four coated polysaccharide-based CSPs were screened in HPLC and SFC modes for the enantioseparation of CLD. The ADMPC-coated chiral stationary phase (CSP) showed the best performance for the enantioseparation. Polar organic mobile phase (ACN/water = 97:3, v/v) is practical for preparative chromatography because of the advantages of high resolution of the enantiomers and the high solubility of (±)-CLD. In SFC, the modifier of methanol exhibits the best chiral

resolution. The chiral resolution is greatly enhanced by the increase of the coating amount of ADMPC under both HPLC and SFC conditions. Semi-preparations were carried out under the conditions of HPLC and SFC with a semi-preparative column packed with 30% ADMPC coated CSP, respectively. The CSP with high ADMPC chiral selector would be more favorited for the enantio-preparation purpose, which can lead to high sample loading capacity, high separation efficiency and low solvent consuming.

## ACKNOWLEDGMENT

This work was supported by the National Natural Science Foundation of China (grant no. 21375038).

## Declarations of interest

None.

## ORCID

*Yanxiong Ke* https://orcid.org/0000-0002-5412-2869

## REFERENCES

1. Li JH, Zhang HD, Wang ZZ, et al. Acute iloprost inhalation improves right ventricle function in pulmonary artery hypertension: a cardiac magnetic resonance study. *Front Pharmacol*. 2019;9:7.
2. Lievre M, Sitruk-Ware R. Meta-analysis of 200 or 600 mg mifepristone in association with two prostaglandins for termination of early pregnancy. *Contraception*. 2010;81(2):173-174.
3. Kelly RW. Pregnancy maintenance and parturition: the role of prostaglandin in manipulating the immune and inflammatory response. *Endocr Rev*. 1994;15(5):684-706.
4. Pai R, Nakamura T, Moon WS, Tarnawski AS. Prostaglandins promote colon cancer cell invasion; signaling by cross-talk between two distinct growth factor receptors. *FASEB J*. 2003; 17(12):1640-1647.
5. Hata AN, Breyer RM. Pharmacology and signaling of prostaglandin receptors: multiple roles in inflammation and immune modulation. *Pharmacol Ther*. 2004;103(2):147-166.
6. Wang D, Mann JR, Dubois RN. The role of prostaglandins and other eicosanoids in the gastrointestinal tract. *Gastroenterology*. 2005;128(5):1445-1461.
7. Corey EJ, Weinshenker NM, Schaaf TK, Huber W. Stereo-controlled synthesis of dl-prostaglandins F2.Alpha. And E2. *J Am Chem Soc*. 1969;91(20):5675-5677.
8. Faseeha N. Hepatitis B virus infection: an insight into infection outcomes and recent treatment options. *Virus*. 2015;26:1-8.
9. Campian M, Putala M, Sebesta R. Bioactive carbocyclic nucleoside analogues–syntheses and properties of entecavir. *Curr Org Chem*. 2014;18(22):2808-2832.
10. Vostrikov NS, Lobko IF, Valiullina ZR, Miftakhov MS. Synthesis of racemic entecavir. *Mendeleev Commun*. 2017;27(1):12-13.
11. Valiullina ZR, Akhmet'yanova VA, Vostrikov NS, Miftakhov MS. A short synthesis of the carbocyclic core of entecavir from Corey lactone. *Mendeleev Commun*. 2016;26(1):9-10.
12. Doyle MP, Catino AJ. A short stereoselective synthesis of (+)- and (−)-2-oxabicyclo[3.3.0]oct-6-en-3-one by intramolecular carbon-hydrogen insertion catalyzed by chiral dirhodium (II) carboxamidates. *Tetrahedron: Asymmetry*. 2003;14(7):925-928.
13. Kasai S, Nagasawa H, Shimamura M, Uto Y, Hori H. Design and synthesis of antiangiogenic/heparin-binding arginine dendrimer mimicking the surface of endostatin. *Bioorg Med Chem Lett*. 2002;12(6):951-954.
14. Nakashima H, Sato M, Taniguchi T, Ogasawara K. Chiral preparation of polyoxygenated cyclopentanoids. *Synthesis*. 2000; 2000(06):817-823.
15. Nakashima H, Sato M, Taniguchi T, Ogasawara K. Lipase-mediated resolution of cis-4-cumyloxy-2-cyclopenten-1-ol and its utilization for enantioconvergent preparation of (−)-oxabicyclo 3.3.0 -oct-6-en-3-one. *Synlett*. 1999;1999(11):1754-1756.
16. Seiichi T, Tsutomu S. Method for optical resolution of corey lactone diols patent US5219743 (A). 1993 July 15.
17. Yakura T. A short synthesis of optically active Corey lactone by means of the Dirhodium (II)-catalyzed intramolecular C-H insertion reaction of an α-diazo ketone. *Synthesis*. 1998; 1998(07):973-974.
18. Romano A, Romano D, Molinari F, Gandolfi R, Costantino F. A new chemoenzymatic synthesis of D-cloprostenol. *Tetrahedron Asymmetry*. 2005;16(19):3279-3282.
19. Szanya T, H-Veszprem, Hanak L. Process for the separation of optical isomers of the corey lactone patent WO2007066160/A1. 2007 June 14.
20. Ikai T, Okamoto Y. Structure control of polysaccharide derivatives for efficient separation of enantiomers by chromatography. *Chem Rev*. 2009;109(11):6077-6101.
21. Yamamoto C, Yamada K, Motoya K, et al. Preparation of HPLC chiral packing materials using cellulose tris(4-methylbenzoate) for the separation of chrysanthemate isomers. *J Polym Sci, Part A: Polym Chem*. 2010;44:5087-5097.
22. Yashima E, Sahavattanapong P, Okamoto Y. HPLC enantioseparation on cellulose tris(3,5-dimethylphenylcarbamate) as a chiral stationary phase: influences of pore size of silica gel, coating amount, coating solvent, and column temperature on chiral discrimination. *Chirality*. 2015;8:446-451.
23. Subramanian G. *Chiral Separation Techniques: A Practical Approach*. Weinheim: Wiley-VCH; 2001:199.
24. Chiyo Y, Eiji Y, Yoshio O. Structural analysis of amylose tris(3,5-dimethylphenylcarbamate) by NMR relevant to its chiral recognition mechanism in HPLC. *J Am Chem Soc*. 2002;124:12583-12589.
25. Li WZ, Wang X, Zhang WQ, et al. Enantioseparation of novel chiral tetrahedral clusters on an amylose tris-(3,5-dimethylphenylcarbamate) chiral stationary phase by normal phase HPLC. *Chin J Chem*. 2004;22:1070-1074.
26. Gao J, Qu H, Zhang C, Li W, Wang P, Zhou Z. Direct chiral separations of the enantiomers of phenylpyrazole pesticides and the metabolites by HPLC. *Chirality*. 2017;29(1):19-25.

27. Shamsipur M, Heydari R, Jamasbi ES, Abdollahpour A. Chiral separation and quantitation of dorzolamide hydrochloride enantiomers by high-performance liquid chromatography. *J Sep Sci*. 2010;33(15):2328-2333.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**How to cite this article:** Wang H, Wang Q, Wu Y, et al. HPLC and SFC enantioseparation of (±)-Corey lactone diol: Impact of the amylose tris-(3,5-dimethylphenylcarbamate) coating amount on chiral preparation. *Chirality*. 2019;1–10. https://doi.org/10.1002/chir.23118