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Application and comparison of high-speed countercurrent chromatography and high performance liquid chromatography in preparative enantioseparation of α -substitution mandelic acids

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Abstract

Preparative enantioseparations of α -cyclopentylmandelic acid and α -methylmandelic acid by high-speed countercurrent chromatography (HSCCC) and high performance liquid chromatography (HPLC) were compared using hydroxypropyl- β -cyclodextrin (HP- β -CD) and sulfobutyl ether- β -cyclodextrin (SBE- β -CD) as the chiral mobile phase additives. In preparative HPLC the enantioseparation was achieved on the ODS C₁₈ reverse phase column with the mobile phase composed of a mixture of acetonitrile and 0.10 mol L⁻¹ phosphate buffer at pH 2.68 containing 20 mmol L⁻¹ HP- β -CD for α -cyclopentylmandelic acid and 20 mmol L⁻¹ SBE- β -CD for α -methylmandelic acid. The maximum sample size for α -cyclopentylmandelic acid and α -methylmandelic acid was only about 10 mg and 5 mg, respectively. In preparative HSCCC the enantioseparations of these two racemates were performed with the two-phase solvent system composed of *n*-hexane-methyl *tert*.-butyl ether-0.1 mol L⁻¹ phosphate buffer solution at pH 2.67 containing 0.1 mol L⁻¹ HP- β -CD for α -cyclopentylmandelic acid (8.5:1.5:10, v/v/v) and 0.1 mol L⁻¹ SBE- β -CD for α -methylmandelic acid (3:7:10, v/v/v). Under the optimum separation conditions, total 250 mg of racemic α -cyclopentylmandelic acid could be completely enantioseparated by HSCCC with HP- β -CD as a chiral mobile phase additive in a single run, yielding 105-110 mg of enantiomers with 95-98% purity and 85-90% recovery. But, no complete enantioseparation of α -methylmandelic acid was achieved by preparative HSCCC with either of the chiral selectors due to their limited enantioselectivity. In this paper preparative enantioseparation by HSCCC and HPLC was compared from various aspects.

Keywords

Preparative enantioseparation; High-speed countercurrent chromatography; High performance liquid chromatography; α -Cyclopentylmandelic acid; α -Methylmandelic acid

1. Introduction

In the past several years, preparative chromatographic enantioseparation of racemates into their individual enantiomers has become an integral part of procedures for screening new drug entities. The overwhelming majority of chemical structures for new drug candidates tend to be asymmetrical where these enantiomers generally possess different activity and toxicity. To obtain a small amount of enantiomer with high optical purity within a short period of time is critical for the initial stages of new drug development. Among the various kinds of chiral separation methods, preparative chromatography, especially preparative high performance liquid chromatography (HPLC), plays a dominant role in the modern pharmaceutical engineering. However, preparative HPLC method is a rather expensive technique, compared to traditional purification methods such as distillation, crystallization or extraction, and it has been used only for rare or expensive products and it becomes much more expensive when it is used for chiral separation because chiral stationary phase or chiral mobile phase is essential for enantioseparations. Usually the cost for chiral HPLC column is rather higher than chiral mobile phase additive method. Therefore, with the increasing demand for production of compounds with high optical purity, it becomes urgent to develop a cost-effective preparative chromatographic method for the chiral separation.

High-speed countercurrent chromatography (HSCCC), which was developed in the end of 1970s, has become a popular modern technique for effective preparative separations of components from natural products, synthetic mixtures and ferment broths [1-3]. Since HSCCC uses a liquid stationary phase without solid support, sample loss due to irreversible adsorption onto the column is avoided. The distinctive characteristics for HSCCC lies in its preparative capacity due to its ease to be scaled up compared with conventional HPLC, and it is especially useful for preparative enantioseparations. Unfortunately, HSCCC has not been a mainstream technique for chiral chromatography due to its low theoretical plates of the separation column. Only very limited number of literatures are available in the past few years [4-6]. Generally, high enantioseparation factor, e.g., greater than 1.4 was necessary for complete resolution of enantiomers, but enantioselectivity generally tends to be lower than 1.4 for common enantiomers. Furthermore, chiral enantioselectivity is greatly affected by different type of chiral selector and the biphasic solvent systems, e.g., chiral selector dissolved in solvent systems might be inactivated by solvation effect. Therefore, successful enantioseparation by HSCCC could be only achieved when a chiral selector with high enantioselectivity along with a suitable biphasic solvent system is used. In our previous studies, hydroxypropyl- β -cyclodextrin (HP- β -CD) was successfully used as the chiral selector for enantioseparation of several aromatic acids by HSCCC [7-11]. During our recent studies, HP- β -CD and sulfobutyl ether- β -cyclodextrin (SBE- β -CD) were used as the chiral mobile phase additive to enantioseparate ten different mandelic acid derivatives by HPLC [12], in which eight racemates were successfully enantioseparated. Since only very small amount of sample could be enantioseparated by preparative HPLC, HSCCC was employed as an alternative separation method for a larger scale preparative separation. It was found that HSCCC could successfully enantioseparate much higher amount of the target compounds with the same chiral selector. But, as we expected, no successful enantioseparation was obtained for the racemate with a low enantioseparation factor. The

present paper reports the application and comparison of these two separation techniques for preparative enantioseparation of two α -substitution mandelic acids, α -cyclopentylmandelic acid and α -methylmandelic acid, whose chemical structures are shown in Fig. 1.

2. Experimental Section

2.1. Apparatus

A type-J high-speed countercurrent chromatography apparatus was used (Model TBE 200VA, Shanghai Tauto Biotechnology, Shanghai, China). It was equipped with three upright multilayer coil separation columns connected in series. Each column consists of 1.6 mm ID PTFE tubing with a total capacity of 190 mL. Detailed parameters of the above apparatus had been reported in our previous paper [7]. The HSCCC centrifuge was placed in a vessel that maintains column temperature by a Model SDC-6 constant-temperature controller (Ningbo Scientz Biotechnology Co. Ltd., Ningbo, China). The solvents were pumped into the column with a model s-1007 constant-flow pump (Beijing Shengyitong Technique, Beijing, China). Continuous monitoring of the effluent was achieved with a model UVD-200 UV detector (Shanghai Jinda Biotechnology Co., Ltd., Shanghai, China) and SEPU3010 workstation (Hangzhou Puhui Technology, Hangzhou, China) was employed to record the chromatogram. The analytical high-performance liquid chromatography (HPLC) used was a CLASS-VP Ver.6.1 system (Shimadzu, Japan) comprised of a Shimadzu SPD10Avp UV detector, a Shimadzu LC-10ATvp Multisolvent Delivery System, a Shimadzu SCL-10Avp controller, a Shimadzu LC pump, and a CLASS-VP Ver.6.1 workstation. The preparative HPLC used was a Knauer chromatographic system (Knauer, Germany) comprised of a Knauer K-2501 UV detector, two Knauer K-501 HPLC pumps and an EastChrom Plus workstation. The sample loop for preparative HPLC was 2 mL. The pH value was determined with a portable Delta 320-s pH meter (Mettler-Toledo, Greifensee, Switzerland).

2.2. Reagents

Racemic α -cyclopentylmandelic acid was purchased from J&K Scientific Ltd., Shanghai, China and α -methylmandelic acid from Tokyo Chemical Industry Co, Ltd., Tokyo, Japan. HP- β -CD and SBE- β -CD (degree of substitution 7.5 and 6.9, respectively) were purchased from Qianhui Fine Chemical & Co. Inc., Shandong, China. Sodium dihydrogen phosphate, phosphoric acid, acetic acid and sodium acetate with analytical grade were all purchased from local commercial chemical store. Acetonitrile and methanol used for HPLC were of a chromatographic grade. Water used for HPLC study was redistilled.

2.3. HPLC separation

2.3.1 Analytical method—The quantification of enantiomers was performed by the Shimadzu HPLC system using a UV detector at 220 nm. The separation was performed on the Shimpak CLC-ODS (150×4.6 mm i.d., 5 μ m) column. The mobile phase was a mixture of acetonitrile and 0.1 mol L⁻¹ phosphate buffer (pH 2.68 adjusted with phosphoric acid) containing 20 mM of HP- β -CD (40:60, v/v) for α -cyclopentylmandelic acid and 20 mM of SBE- β -CD (10:90, v/v) for α -methylmandelic acid. The flow rate was 0.6 mL min⁻¹. The

mobile phase was filtered through a 0.45 μm filter and sonicated for 20 min prior to use. The column temperature was set at 25°C.

2.3.3. Preparative method—The preparative enantioseparation of α -substitution mandelic acids enantiomers was performed by the Knauer HPLC system using a UV detector set at 220 nm. The column was Venusil XBP C₁₈, with 10 μm particle size of the packing material, 250 mm $\times$ 10 mm I.D. (Bonna-Agela Technologies Co., Ltd., Wilmington, USA). Composition of the mobile phase for α -cyclopentylmandelic acid separation was identical to that of analytical HPLC, and for α -methylmandelic acid separation, the mobile phase was identical with that of analytical HPLC but with different volume ratio (5:95, v/v). A flow rate of 1.0 mL min⁻¹ was used for all separation. The column temperature was set at 15°C.

2.4. HSCCC separation

2.4.1. Enantioselective liquid-liquid extractions—Determinantion of the distribution ratio of enantiomers is essential for selection of the solvent system prior to HSCCC separation. Distribution ratios for enantiomers were calculated by the concentration of enantiomer in the organic phase divided by that in the aqueous phase. The quantitative distribution of racemate in the biphasic solvent system was determined by means of liquid-liquid extraction experiments under 10°C. The organic/aqueous solvent systems were prepared in advance and allowed to equilibrate over 2 h. Two milliliters of the organic phase and two milliliters of the aqueous phase containing 0.1 mol L⁻¹ of chiral selector and 2 mmol L⁻¹ of racemate were delivered into a 10 mL glass tube. After capping, the tube was shaken vigorously for 10 min to equilibrate the sample in two phases. The distribution of enantiomer in each phase was analyzed by HPLC. The influence factors including concentration of chiral selector, pH value of the aqueous phase and equilibrium temperature were further investigated with liquid-liquid extraction experiments, using the molar ratio of chiral selector/racemate at 50:1.

2.4.2. Preparation of HSCCC solvent systems and sample solutions—The present studies were performed with two-phase solvent systems composed of *n*-hexane-methyl *tert*.-butyl ether-0.1 mol L⁻¹ phosphate buffer solution at pH 2.67 containing 0.1 mol L⁻¹ chiral selector at the volume ratio of 8.5:1.5:10 for α -cyclopentylmandelic acid separation and 3:7:10 for α -methylmandelic acid separation. The aqueous buffer solution at pH 2.67 was prepared before adding 0.1 mol L⁻¹ chiral selector. Then the solvent mixture was thoroughly equilibrated in a separatory funnel at 10°C in a water bath, and the two phases were separated and degassed by ultrasound for 30 min shortly before use. Temperature was controlled at 10°C for mobile aqueous phase in a water bath during CCC separation. The sample solutions were prepared as follows: 250 mg of racemic α -cyclopentylmandelic acid was dissolved in 20 mL of the organic phase and 8 mg of racemic α -methylmandelic acid was dissolved in 4 mL of the organic phase for preparative separation.

2.4.3. HSCCC separation—The preparative separations were initiated by filling the column with the organic stationary phase. The aqueous mobile phase was pumped into the column in the head to tail elution mode while the column was rotated at 800 rpm. Each of

the sample solution was injected after the hydrodynamic equilibrium was reached, as indicated by a clear mobile phase eluting at the tail outlet. The effluent from the outlet of the column was continuously monitored at 254 nm. Temperature was controlled at 10°C during separation.

2.5. Recovery of solutes from chiral CCC fractions

Because the fractions from preparative HPLC and HSCCC contained chiral selectors, recovery of the α -cyclopentylmandelic acid enantiomer from the separated fractions was necessary: Each collected fraction containing the enantiomers were acidified with a small volume of concentrated hydrochloric acid and extracted three times with ethyl acetate. The combined organic layers were dried with anhydrous sodium sulfate and filtered, and the solvent was evaporated under reduced pressure. The residue of the organic layers was spotted on silica gel TLC plates and developed with chloroform: *n*-hexane: glacial acetic acid (5:5:0.05, v/v/v). The visual detections were done by concentrated sulfuric acid vapor. The experimental results showed that R_f value of α -cyclopentylmandelic acid enantiomer spots on the TLC was 0.38, R_f values of HP- β -CD was less than 0.05. In order to purify the enantiomers, the residue was further subjected to the silica gel column chromatography with isocratic elution (chloroform: *n*-hexane: glacial acetic acid=5:5:0.05) to remove the small amount of cyclodextrins. Recovery of α -methylmandelic acid was not investigated.

3. Results and discussion

3.1. Enantioseparation of α -substitution mandelic acids by preparative HPLC

in our previous studies enantioseparation of two α -substitution mandelic acids was performed using a conventional ODS C₁₈ HPLC analytical with HP- β -CD or SBE- β -CD as chiral mobile phase additives [12], in which the mobile phase was a mixture of acetonitrile and 0.10 mol L⁻¹ of phosphate buffer at pH 2.68 containing 20 mmol L⁻¹ of chiral additives. α -Cyclopentylmandelic acid could be enantioseparated by analytical HPLC when HP- β -CD or SBE- β -CD was used as a chiral mobile phase additive. HP- β -CD showed higher enantio recognition for α -cyclopentylmandelic acid and thus provided much higher peak resolution than SBE- β -CD, whereas α -methylmandelic acid could only be enantioseparated with SBE- β -CD as the chiral mobile phase additive.

The organic modifier added in the mobile phase played a key role in the enantioseparations. Our results showed that much longer retention time was needed for methanol than acetonitrile for base line separation of the enantiomers, e.g., with the mobile phase consisting of phosphate buffer at pH 2.68 containing 10 mmol L⁻¹ of HP- β -CD and organic modifier (60:40) for the enantioseparation of α -cyclopentylmandelic acid, the retention time was 16.0-17.0 min when acetonitrile was used as the organic modifier whereas 59.3-68.0 min was needed to elute the enantiomers with slightly higher resolution with the methanol used as the organic modifier. Thus, acetonitrile was preferred for the analytical chiral HPLC separations. However, for preparative HPLC enantioseparations, the method should be focused on injecting maximum amount of samples to yield moderate peak resolution. Taking the racemic α -cyclopentylmandelic acid as an example, its solubility in the mobile phase of HPLC is critically related to the percentage of organic solvent. The higher is the percentage

of the organic solvent, the higher solubility of the sample will be obtained, while increasing the percentage of organic modifier in the mobile phase would lead to lower peak resolution. Thus, in order to get an ideal enantioseparation results for preparative HPLC, an optimal percentage point of organic solvent was determined, in which the mobile phase consisting of phosphate buffer at pH 2.68 containing 20 mmol L⁻¹ of HP- β -CD and organic modifier (60:40) was found to be the best volume ratio for enantioseparation of α -cyclopentylmandelic acid. On the other hand, the racemic α -cyclopentylmandelic acid showed higher solubility in the mobile phase containing acetonitrile than methanol as an organic modifier. Under 15°C, the maximum solubility for α -cyclopentylmandelic acid in the mobile phase modified by acetonitrile was about 20 mg mL⁻¹ with aqueous phase : acetonitrile (60:40, v/v) and it decreased to 7 mg mL⁻¹ with aqueous phase : acetonitrile (70:30, v/v), whereas solubility was only about 8 mg mL⁻¹ in the mobile phase with methanol as an organic modifier with aqueous phase : methanol (60:40, v/v). Thus, acetonitrile was also selected as the organic modifier for the mobile phase for preparative HPLC enantioseparations. Fig. 2(a) shows a typical chromatogram for enantioseparation of racemic α -cyclopentylmandelic acid by the preparative HPLC column with HP- β -CD as a chiral mobile phase additive. The maximum sample loading capacity was only about 10 mg of racemate which yielded the peak resolution $R_s=0.59$. The mobile phase was composed of phosphate buffer at pH 2.68 containing 20 mmol L⁻¹ of HP- β -CD and acetonitrile (60:40, v/v).

Under temperature at 15°C, the effect of flow rate on the separation was investigated in the range of 1.0-3.0 mL min⁻¹. Considering both retention time and peak resolution, a flow rate of 1.0 mL min⁻¹ was selected. Each peak fraction was manually collected according to the chromatogram. As for preparative enantioseparation of α -methylmandelic acid, only 5 mg of racemate could be partially enantioseparated with SBE- β -CD as a chiral selector under optimized chromatographic conditions. The mobile phase was composed of phosphate buffer at pH 2.68 containing 20 mmol L⁻¹ of SBE- β -CD and acetonitrile (95:5, v/v).

The preparative HPLC fractions of α -cyclopentylmandelic acid were analyzed by analytical HPLC. Results showed that the purity of α -cyclopentylmandelic acid enantiomers was over 95%. Recovery of these enantiomer was in the range of 66-85% and 3-4 mg of enantiomer were obtained from the preparative enantioseparation.

3.2. Enantioseparation of α -substitution mandelic acids by preparative HSCCC

In enantioseparation by HSCCC, a suitable two-phase solvent system along with the effective chiral selector need to be selected. Generally, the solvent system used in enantioseparation by HSCCC needs to satisfy the following requirements: the analytes to be separated could partition at a suitable ratio between the two phases, and the chiral selector is mainly distributed in one phase of the solvent system. In addition the solvent system should not disrupt its chiral recognition for enantiomers. These requirements play a key role in successful enantioseparation by HSCCC. The chiral selector used in HSCCC was generally derived from other techniques such as chiral liquid chromatography and electrophoresis. HP- β -CD shows high solubility in the water, which indicates high enantiorecognition in the aqueous solutions and it is a good chiral selector for some aromatic acids. It is worthy of

pointing out that enantiorecognition ability of HP- β -CD in the aqueous solution might completely disappear if some hydrophilic organic solvent such as methanol was added in the aqueous solution. The main reason for this phenomenon is the following: chiral recognition of HP- β -CD results from formation of the inclusion complex between host and guest, in which several weak intermolecular forces such as dipole-dipole, hydrophobic, Van der Waals, electrostatic, and hydrogen bonding interaction, cooperatively contribute to the formation of diastereomeric complexes between enantiomers and HP- β -CD, which enable HP- β -CD to recognize enantiomers. Methanol molecule can enter the hydrophobic cavity of HP- β -CD to interfere with the formation of inclusion complex, hence no enantiorecognition could be attained for enantiomers. And because of this, the selection of the biphasic solvent system for CCC enantioseparation with HP- β -CD as chiral selector becomes restricted to water free of any hydrophilic organic solvent whereas the selection of the organic phase become much more flexible and is largely dependent on target analytes. On the other hand, analyte with poor solubility in the water might show improved solubility in the aqueous phase when certain concentration of cyclodextrin is added to the aqueous phase due to the formation of inclusion complex. In brief distribution ratio for enantiomers should be tuned by changing the composition of organic phase so that the distribution ratio of the target component falls within a suitable range (i.e. usually between 0.2 and 5.0) for the HSCCC separation.

Several two-phase solvent systems were examined under 10°C and their distribution ratios of α -substitution mandelic acids enantiomer were measured. All the aqueous phases used in the following two-phase solvent systems were 0.1 mol L⁻¹ phosphate buffer solution at pH 2.67 added with 0.1 mol L⁻¹ of HP- β -CD or SBE- β -CD. The two-phase solvent systems composed of *n*-hexane-methyl *tert*-butyl ether-aqueous phase with various volume ratios were tested. At the same time, influence factors including concentration of HP- β -CD, separation temperature and pH value were further investigated in order to improve the enantioseparation factor. The experimental results showed that the optimum conditions for enantioseparation of two α -substitution mandelic acids were quite similar to the results obtained in our previous studies [8-10]. Briefly, low temperature (5-10°C) and low pH value (less than 3.0) of the aqueous phase led to higher enantioselectivity when the concentration of chiral selector was in the range of 0.05-0.10 mol L⁻¹. Table 1 shows results for determination of the distribution ratio and enantioselectivity of the two racemates. Unfortunately, only HP- β -CD gave high enantioselectivity (α =1.750) for enantiomers of α -cyclopentylmandelic acid. As for α -methylmandelic acid, both of enantioselectivity were about 1.1 with either chiral selector, which was too small for successful enantioseparation by HSCCC. Although moderate resolution of α -methylmandelic acid could be obtained by preparative HPLC with SBE- β -CD as the chiral mobile phase additive, no resolution could be achieved by HSCCC with SBE- β -CD as the chiral selector.

As for preparative enantioseparation by HSCCC, the maximum amount sample to be injected into the column was generally determined by the following two aspects: the solubility of the sample in the biphasic solvent systems and the amount of chiral selectors in the column. Taking the racemic α -cyclopentylmandelic acid as an example, the solubility was about 12 mg mL⁻¹ in the organic phase and 3 mg mL⁻¹ in the aqueous phase of the

biphasic solvent system. The sample can be dissolved in either phase used for separation. Since the sample loop was 20 mL, total 240 mg of racemate could be injected if the sample was dissolved in the organic phase. One of the most attracting advantages for HSCCC separation was about its flexible preparation of sample solutions. Though the maximum solubility for racemic α -cyclopentylmandelic acid in the solvent system was 12 mg mL^{-1} , the sample solutions containing undissolved sample could also be injected without filtration, which means a large amount of sample could be injected into the column regardless of its solubility. Fig. 3 shows a typical chromatogram for enantioseparation of 250 mg of racemic α -cyclopentylmandelic acid by HSCCC. The sample solution injected was a suspension, but it didn't affect its resolution, and almost base line separation was achieved. It was performed with head-to-tail elution mode, in which aqueous phase was used as the mobile phase with HP- β -CD as the chiral mobile phase additive. The molar ratio of HP- β -CD/racemate was 6:1 in this chromatographic system. Generally, the phase which contains the chiral selector was preferred to be the stationary phase for enantioseparation by HSCCC. However, it was found that peak resolution was much lower when the aqueous phase was used as stationary phase. On the other hand, better comparison between preparative HPLC and HSCCC could be observed if both of the techniques were conducted with chiral mobile phase additive method. Recovery of both enantiomers of α -cyclopentylmandelic acid was in the range of 85-90% and 105-110 mg of enantiomers were obtained from the preparative separation.

For preparative enantioseparation of α -methylmandelic acid by HSCCC with either HP- β -CD and SBE- β -CD used as chiral mobile phase additive, no peak resolution was observed. Fig. 4(a) presented the chromatogram for enantioseparation of 8 mg of α -methylmandelic acid by HSCCC with SBE- β -CD as chiral selector. Analytical chiral HPLC was used to evaluate the enantiomeric content of the eluted fractions from HSCCC and Fig. 4(b) shows the elution profiles. Where only slight resolution was obtained for racemic α -methylmandelic acid.

3.3. Comparison of HPLC and HSCCC in preparative enantioseparation

Generally, peak resolution, retention time and sample loading are the three key parameters that need to be considered for preparative chromatography. These parameters are critically dependent on each other. Peak resolution is determined by column efficiency with a given amount of sample loading. The number of theoretical plates (N) for separation column is generally employed for comparison. The number of theoretical plates for symmetrical peak, as generally produced in analytical chromatography, could be calculated by Gaussian peak dispersion equation. But most of the peaks emerged in separations by preparative chromatography were not so ideal and skewed and irregular peaks were frequently observed. The number of theoretical plates for an asymmetrical peak could be evaluated by EMG method (exponentially modified Gaussian) [13]:

$$N = \frac{41.7(t_R/W_{0.1})^2}{B/A + 1.25} \quad (1)$$

where t_R is the retention time, $W_{0.1}$ means the peak width at 10% peak height and B/A is the empirical asymmetrical factor.

The capacity factor k' for HSCCC could be calculated by the following equation:

$$k' = D \left(\frac{S_F}{1 - S_F} \right) \quad (2)$$

$$D = \frac{V_R - V_m}{V_C - V_m} \quad (3)$$

where D is the distribution ratio and S_F is the ratio between the volume of stationary phase and column volume, and V_R is the retention volume of the enantiomer, and V_C and V_m are the of the column volume and volume of the mobile phase in the column, respectively.

Preparative HPLC generally yields higher separation efficiency than HSCCC. A satisfactory peak resolution could still be obtained with rather low separation factor, e.g., α 1.1. But it is impossible for HSCCC to give a complete separation if the separation factor was less than 1.4, which makes it difficult in application of enantioseparations since most of the enantioselectivity was very low. No peak resolution was observed for enantioseparation of α -methylmandelic acid by HSCCC with SBE- β -CD as a chiral selector, but preparative HPLC provided satisfactory peak resolution for α -methylmandelic acid with the same chiral selector as the mobile phase additive.

For enantioseparation of α -cyclopentylmandelic acid with HP- β -CD as chiral mobile phase additive, both methods could give satisfactory peak resolutions, but preparative HSCCC showed a much greater preparative capacity than preparative HPLC. A comparison of preparative HPLC and HSCCC used in enantioseparation of α -cyclopentylmandelic acid was summarized in Table 2. Sample loading limits for preparative HPLC with HP- β -CD as chiral mobile phase additive was 10 mg with moderate resolution while it could reach 250 mg of racemate injected in preparative HSCCC with higher resolution. Though total solvent and total amount of chiral selector consumed by HSCCC were higher than HPLC, its unit productivity (mg min^{-1}), unit solvent consumption (mL mg^{-1}) and unit chiral selector consumption (mmol mg^{-1}) were much lower than HPLC. The above comparison demonstrated that HSCCC could show higher efficiency than HPLC as long as enantioseparation factor in the chromatographic system was high enough. On the other hand, generally HPLC needs reagent of chromatographic grade while HSCCC needs reagent of only analytical or chemical grade. As for the stationary phase, a conventional stationary phase column is necessary for HPLC separation while only solvent could be used as the stationary phase for HSCCC. Thus, generally higher cost for preparative enantioseparation by HPLC was necessary than that of HSCCC. Finally, as expected, theoretical plates for HPLC column is generally higher than that of HSCCC.

4. Conclusions

Enantioseparations of α -substitution mandelic acids were investigated by preparative HPLC and HSCCC with HP- β -CD or SBE- β -CD as the chiral mobile phase additives. Optimization of mobile phase for preparative HPLC and the biphasic solvent systems for preparative HSCCC were accomplished. Successful enantioseparations of α -cyclopentylmandelic acid

and α -methylmandelic acid were achieved by preparative HPLC with HP- β -CD and SBE- β -CD as chiral mobile phase additive, respectively. However, only α -cyclopentylmandelic acid could be completely enantioseparated by HSCCC with HP- β -CD as chiral mobile phase additive. No successful separation was achieved for α -methylmandelic acid by HSCCC due to its low enantioselectivity. Under optimum separation conditions, the maximum sample loading was determined for each separation method. Comparison of preparative HPLC and HSCCC was evaluated concerning chromatographic parameters, productivity, solvent and chiral selector consumptions. The above results showed that HSCCC could be selected as highly efficient enantioseparation alternative method with high capacity but lower cost, if higher enantioseparation factor was available for enantiomers.

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Fig. 1.
chemical structures of α -substitution mandelic acids

Fig. 2.

Preparative HPLC chromatogram for enantioseparation of (a) α -cyclopentylmandelic acid and (b) α -methylmandelic acid. Column: Venusil XBP C18 (250 mm $\times$ 10 mm I.D.); mobile phase: (a) phosphate buffer at pH 2.68 containing 20 mmol L⁻¹ of HP- β -CD and acetonitrile (60:40, v/v) and (b) phosphate buffer at pH 2.68 containing 20 mmol L⁻¹ of SBE- β -CD and acetonitrile (95:5, v/v); flow rate: 1.0 mL min⁻¹; column temperature: 15°C.

Fig. 3.

Chromatogram of preparative enantioseparation of α -cyclopentylmandelic acid by HSCCC. Solvent system: *n*-hexane : methyl *tert*-butyl ether : 0.1 mol L⁻¹ phosphate buffer pH2.68 (8.5:1.5:10, v/v/v); stationary phase: upper organic phase; mobile phase: lower aqueous phase; sample solution: 250 mg of racemic α -cyclopentylmandelic acid dissolved in 20 mL of the organic phase; flow rate: 2.0 mL min⁻¹; revolution: 800 rpm; column temperature: 10 °C; stationary phase retention: 67.5%.

Fig. 4.

(a) Chromatogram of preparative enantioseparation of α -methylmandelic acid by HSCCC. Solvent system: *n*-hexane : methyl *tert*-butyl ether : 0.1 mol L⁻¹ phosphate buffer pH2.68 (3:7:10, v/v/v); stationary phase: upper organic phase; mobile phase: lower aqueous phase; sample solution: 8 mg of racemic α -methylmandelic acid dissolved in 4 mL of the organic phase; flow rate: 1.0 mL min⁻¹; revolution: 800 rpm; column temperature: 10 °C; stationary phase retention: 72.5%. (b) Elution profiles for (a) determined by analytical HPLC. For analytical HPLC experimental conditions, see experimental section.

Table 1

distribution ratio and enantioselectivity of the two racemates with different chiral selector

Racemate	Chiral selector	D_+	D_-	α
α -methylmandelic acid	HP- β -CD	2.728	3.067	1.124
	SBE- β -CD	2.392	2.739	1.145
α -cyclopentylmandelic acid	HP- β -CD	0.676	1.183	1.750
	SBE- β -CD	0.818	0.864	1.056

Organic phase: *n*-hexane-methyl tert-butyl ether (8.5:1.5 for α -methylmandelic acid and 3:7 for α -cyclopentylmandelic acid, v/v); aqueous phase: phosphate buffer solution pH2.68 containing 2 mmol L⁻¹ α -substitution mandelic acids and 0.10 mol L⁻¹ chiral selector and equilibrium temperature: 5 °C.

Table 2Comparison of HPLC and HSCCC in enantioseparation of α -cyclopentylmandelic acid

	Preparative HPLC	Preparative HSCCC
Separation column	250 mm $\times$ 10 mm I.D. ODS C ₁₈	1.6 mm ID PTFE tubing (190mL)
Mobile phase flow rate (mL min ⁻¹)	1	2
Sample loading limits (mg)	10	250
Column temperature (°C)	15	10
Run time (min)	100	180
Theoretical plates <i>N</i>	1094	134
Resolution <i>R_S</i>	0.59	0.89
Total mobile phase used (mL)	160(chromatographic grade)	400 (analytical grade)
Total stationary phase used (mL)	N/A	200 (analytical grade)
Total solvent used (mL)	160(chromatographic grade)	600 (analytical grade)
Total chiral selector used (mmol)	3.2	18.0
Productivity (mg min ⁻¹)	0.10	1.39
Solvent consumption (mL mg ⁻¹)	16	4
Chiral selector consumption (mmol mg ⁻¹)	0.32	0.072
Purity of enantiomers (%)	95%	95%