Tetrahedron: *Asymmetry* 27 (2016) 301–306

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

Enantioseparation of (*RS*)-ibuprofen by closed recycling high-speed counter-current chromatography using hydroxypropyl-β-cyclodextrin as chiral selector

Liya Rong [a], Qi Liu [a,b], Jun Wang [a], Hualiang Zeng [a], Hua Yang [a], Xiaoqing Chen [a,*]

[a] College of Chemistry and Chemical Engineering, Central South University, Changsha 410083, China
[b] China Division of Physical Biology & Bioimaging Center, Shanghai Synchrotron Radiation Facility, Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai, China

ARTICLE INFO

Article history:
Received 23 December 2015
Revised 26 February 2016
Accepted 1 March 2016
Available online 14 March 2016

ABSTRACT

High-speed counter-current chromatography combined with closed recycling elution mode was developed to enantioseparate (*RS*)-ibuprofen by using hydroxypropyl-β-cyclodextrin (HP-β-CD) as the chiral selector. Key parameters for high-speed counter-current chromatography resolution including the concentration of HP-β-CD, the two-phase solvent system composition, equilibrium temperature, and the pH of aqueous phase were extensively investigated. Under the optimized conditions, the enantiomers of ibuprofen were successfully separated by preparative recycling high-speed counter-current chromatography and the resulting enantiopurity of each enantiomer was over 97.5% as determined by HPLC. Moreover, the recovery for (*R*,*S*)-ibuprofen from high-speed counter-current chromatography fractions was achieved in the range of 82–89%.

1. Introduction

Chirality is an inherent feature, which exerts an obvious influence on the interaction between biologically active compounds and chiral compounds.[1,2] In most cases, the two enantiomers differ remarkably in pharmacological and toxicological properties.[3] Sometimes only one of the enantiomers possesses the desirable therapeutic activity, while the other is completely inactive or even harmful.[4] Therefore, the screening of efficient separation methods for obtaining enantiopure compounds is becoming urgent with the rapidly increasing number of chiral drugs.[5,6] Many novel and impressive separation methodologies, such as crystallization techniques,[7] kinetic resolutions,[8,9] and chiral extractions,[10–12] have been proposed for the separation of racemates. Nevertheless, most of the above methods are more or less limited by high capital cost and operational complexity.

High-speed counter-current chromatography is a liquid–liquid partition chromatographic technique in which the stationary and mobile phases are constituted by two immiscible liquids or solutions.[13–15] This technique greatly increases the loading capacity of the stationary phase compared with HPLC[16] and it is especially suited for preparative separation on the basis of different affinities for one or the other phase.[17,18] Nevertheless, high-speed counter-current chromatography separation tends to require a relatively high enantioseparation factor to resolve enantiomers completely, because high-speed counter-current chromatography usually has no more than 1000 theoretical plates.[19] This difficulty can be overcome by utilizing a recycling elution mode,[20–23] in which the effluent is iteratively pumped into the separation column and the theoretical plates are considerably increased. Meanwhile, the resolution of racemic compounds necessitates the presence of a chiral environment. By adding a suitable chiral selector into the two-phase solvent system, high-speed counter-current chromatography coupled with a recycling elution mode is proven to be effective in separating enantiomers.[24,19,18,25] Herein, a variety of β-cyclodextrin (β-CD) derivatives as chiral selector for high-speed counter-current chromatography separation using recycling elution mode was investigated.

Ibuprofen (Fig. 1) is an important chiral compound that is marketed as a non-steroidal anti-inflammatory drug[26,27] all over the world; the pharmacological research shows that (*S*)-ibuprofen is 160 times more active than the (*R*)-enantiomer, which often causes side effects or toxicity such as gastrointestinal problems.[28,29]

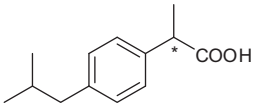

Figure 1. Molecular structure of ibuprofen.

* Corresponding author. Tel.: +86 731 88830833.
E-mail address: xqchen@csu.edu.cn (X. Chen).

http://dx.doi.org/10.1016/j.tetasy.2016.03.001
0957-4166/

Table 1
Comparison of the separation results with existed references related to ibuprofen enantiomers

Methods	Pros	Cons	Refs.
Supercritical fluid chromatography (SFC)	Substantial waste reduction, facilitated product recovery and feasibility of solvent recycling	Limited selectivity of chromatographic column	30
Capillary electrophoresis (CE)	Easy preparation with adjustable porosity and fast diffusional mass transfer	Poor ability for preparation and low recovery	31
Gas chromatography–mass spectrometry (GC–MS)	Separation of the enantiomers without derivatization	Restricted by the temperature	32
Thin layer chromatography (TLC)	Easy operation and simple equipment	Restricted by UV background and chromogenic agents	33
Simulated moving bed (SMB)	Reduced solvent consumption and high separating power	Purity is not high and restricted by adsorbents	34
High-performance liquid chromatography (HPLC)	High sensitivity	Not easy to preparatively separate	35

Although research concerning the separation of the enantiomers of ibuprofen is available (Table 1),[30–35] techniques for large-scale production purposes remain to be studied further. Herein, a method for the preparative separation of pure ibuprofen enantiomers was performed by using closed recycling high-speed counter-current chromatography with hydroxypropyl-β-CD (HP-β-CD) as the chiral selector. The main factors affecting enantioseparation efficiency, such as two-phase solvent system composition, concentration of chiral selector, equilibrium temperature, and the pH of the aqueous phase were also investigated.

2. Results and discussion

2.1. Two-phase solvent systems

Generally, a suitable two-phase solvent system should satisfy the following requirements: (i) the solvent system affording an ideal *K*-value in the range of 0.5–2.0 is highly preferable; (ii) a satisfactory separation factor (>1.1) and the retention of stationary phase (S_F > 40%); and (iii) an equal volume of the two phases and a reasonable settling time (<30 s). Furthermore, the sample should be soluble in both phases while the chiral selector should be soluble in only one phase. Herein, several two-phase solvent systems such as cyclohexane-water, *n*-hexane–methanol–water, and *n*-hexane–ethyl acetate–water with different volume ratios were examined and their results are shown in Table 2. In order to meet the above requirements, the solvent system *n*-hexane–ethyl acetate–water with volume ratios of 8:2:10 and 7:3:10 was taken into consideration. However, *n*-hexane–ethyl acetate–water (8:2:10, v/v/v) was excluded because its *K*-values are close to 0.5, thus resulting in shorter retention time. As a result, the remaining one with better enantioseparation efficiency (1.176) and moderate distribution ratios was chosen.

2.2. Effects of important parameters on high-speed counter-current chromatography enantioseparation

The following parameters were further studied for satisfactory enantioseparation by high-speed counter-current chromatography: the type and concentration of the chiral selector, the pH value of the aqueous solution and the equilibrium temperature.

In order to find a highly enantioselective chiral selector, *K*-values and enantioseparation factor for (*RS*)-ibuprofen were measured in *n*-hexane–ethyl acetate–water (7:3:10, v/v/v) with different β-CD derivatives: SBE-β-CD, ME-β-CD, CM-β-CD, HE-β-CD and HP-β-CD (Table 3). The structure of their cavities and their capability of forming hydrogen bonds can be altered by modifying the chiral selectors, resulting in distinguished enantioseparation efficiency. Herein HP-β-CD was employed as the chiral additive in the aqueous phase since it provided the best enantioseparation factor (1.176) along with suitable *K*-values.

The effects of the concentration of HP-β-CD on enantioseparation efficiency and *K*-value are summarized in Figure 2a and b, respectively. The enantioseparation factor was dramatically improved as the HP-β-CD concentration increased. The maximum enantioseparation factor reached 1.176 at a concentration of 100 mM, but it decreased slightly if more HP-β-CD was added. Figure 2b shows that the *K*-value decreased greatly when increasing HP-β-CD concentration in the range of 0–100 mM. Besides, too large *K*-values and a poor solubility of ibuprofen in the aqueous phase were observed with less than 100 mM. It should be noted that the solubility of (*RS*)-ibuprofen in the aqueous phase was also enhanced due to the selective inclusion interaction between HP-β-CD and enantiomers, resulting in overall decreased *K*-values.

As shown in Figure 2c and d, an identical tendency was observed in the variations of enantioseparation factor and *K*-values. Both parameters decreased when increasing pH-value

Table 3
K-Values and enantioseparation factors of (*RS*)-ibuprofen with different chiral selectors

Chiral selector	*K*-Values		Enantioseparation factor
	K_S	K_R	
SBE-β-CD	1.2477	1.1737	1.063
ME-β-CD	1.0958	1.0514	1.042
CM-β-CD	1.2333	1.1482	1.074
HE-β-CD	1.0760	1.0021	1.074
HP-β-CD	1.2859	1.0934	1.176

Table 2
K-Values and enantioseparation factors of (*RS*)-ibuprofen in different solvent systems ([HP-β-CD] = 100 mM, *T* = 10 °C and pH = 2.5)

Solvent systems	*K*-Values		Enantioseparation factor
	K_S	K_R	
Cyclohexane–water 1:1	6.2515	5.6432	1.108
n-Hexane–methanol–water 20:1:19	0.4003	0.3792	1.056
n-Hexane–methanol–water 10:1:9	0.1034	0.1032	1.002
n-Hexane–methanol–water 10:3:7	0.1977	0.1864	1.061
n-Hexane–ethyl acetate–water 9:1:10	0.6949	0.6839	1.016
n-Hexane–ethyl acetate–water 8:2:10	0.6870	0.7578	1.103
n-Hexane–ethyl acetate–water 7:3:10	1.2859	1.0933	1.176

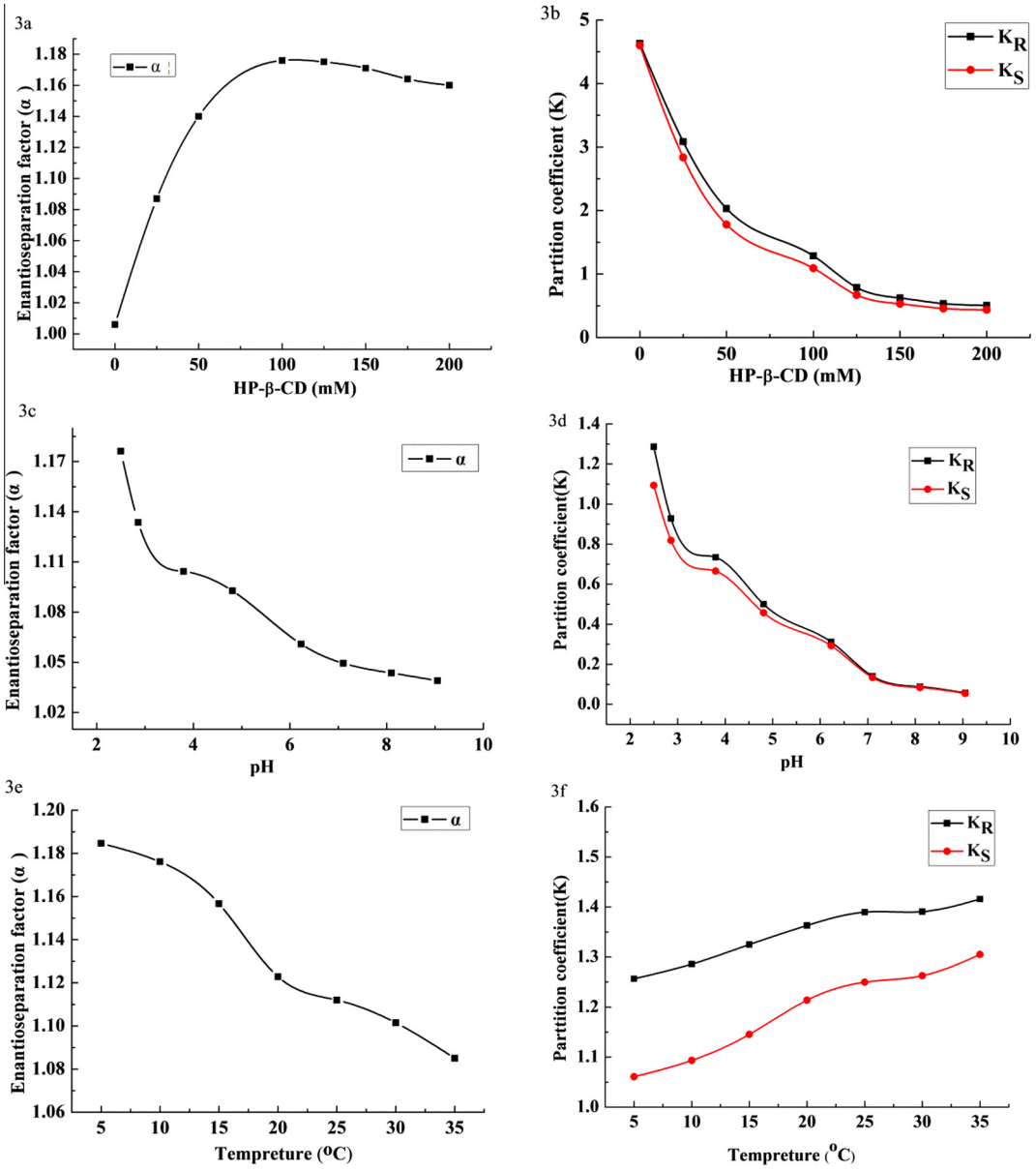

Figure 2. Effects of the different parameters (HP-β-CD concentration, pH, and temperature) on enantioseparation factors and *K*-values for ibuprofen enantiomers.

from 2.50 to 4.81 and then tended to decrease mildly. Ibuprofen mainly exists in the molecular state in the aqueous phase under low pH-values (⩽4.81) while the dissociation of molecular ibuprofen into an ionic form occurs at a higher pH condition (>4.81). In this way, more molecular ibuprofen enantiomers moved from the upper phase to the lower phase. In terms of the enantioseparation factor, the possible reason for this is that HP-β-CD mainly possesses enantio recognition for molecular ibuprofen, rather than for its ionic state. As a result, a pH value of 2.50 was used for the following studies.

The equilibrium temperature plays an important role in enantioseparation for its influences on the partition and solubility of solutes. Figure 2f shows that the *K*-values of both enantiomers increased steadily from 5 °C to 20 °C and remained nearly the same with a further increase in temperature. Nevertheless, the temperature had a negative effect on the enantioseparation factor, which decreased dramatically when increasing temperature (Fig. 2e). As a consequence, higher temperatures weaken the inclusion interactions between HP-β-CD and ibuprofen. The variation of $ln\,\alpha$ against $1/T$ is shown in Figure 3: $ln\,\alpha = 267.19/T - 0.7871$ ($R^2 = 0.9721$), which was fitted well with the Van't Hoff equation. Meanwhile, $\Delta G = -258.23$ J/mol was calculated by using the Gibbs–Helmholtz equation, indicating that the enantio recognition process is spontaneous and the chiral high-speed counter-current chromatography separation should be carried out at a low temperature. Since ibuprofen and HP-β-CD are hardly soluble in the solvent system at 5 °C, an optimal temperature of 10 °C was ultimately selected.

It could be assumed that the current system may give power from the chiral selector in aqueous phase for separation by using monophasic recognition. The diastereoisomeric inclusion complexes can be formed between the ibuprofen enantiomers and HP-β-CD. Due to the presence of steric forces, the inclusion interactions for HP-β-CD and two enantiomers are different. It can be concluded that the stability of the inclusion complex between HP-β-CD and (*R*)-ibuprofen is higher than between (*S*)-ibuprofen according to the determined *K*-values ($K_R < K_S$). Therefore, the chiral separation of the (*R*)- and (*S*)-ibuprofen enantiomers was achieved in the proposed method.

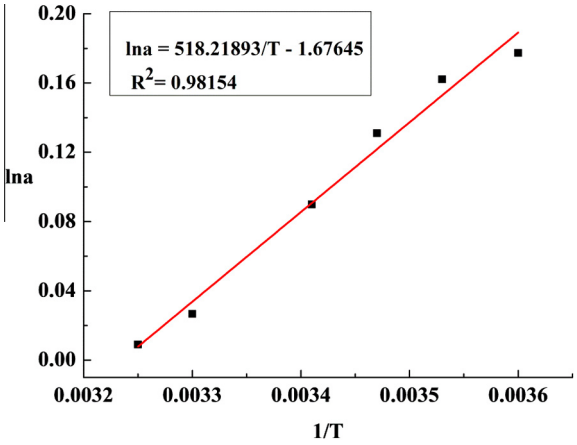

Figure 3. Graph of $ln\,\alpha$ being plotted against $1/T$.

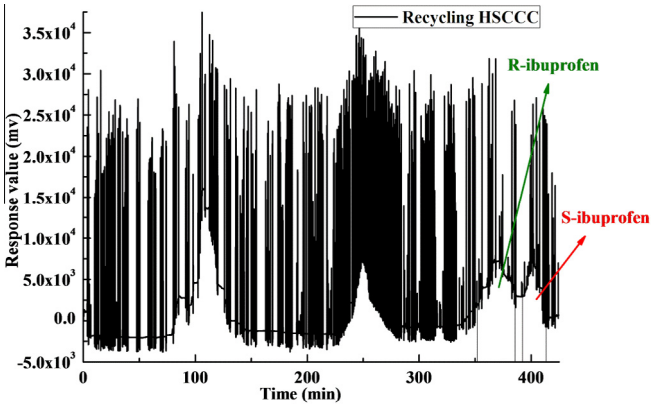

Figure 4. High-speed counter-current chromatography (HSCCC) chromatogram of ibuprofen enantiomers (high-speed counter-current chromatography conditions: revolution speed: 850 rpm; sample loading: 100 mg; detection wavelength: 254 nm; flow rate: 1.2 mL/min; separation temperature: 10 °C; retention of the stationary phase: 53.73%).

2.3. Enantioseparation by high-speed counter-current chromatography and purity analysis

It is difficult to successfully carry out enantioseparation by conventional high-speed counter-current chromatography due to its limited peak capacity and close *K*-values for enantiomers. A recycling elution mode, endowed with the capability of improving separation factor and eliminating the loss of mobile phase and sample, is an ideal alternative. Therefore, recycling high-speed counter-current chromatography with the optimized solvent system was adopted to completely separate (*RS*)-ibuprofen. In most cases, the chiral selector should be added into the stationary phase in order to avoid the loss of chiral selector and obtain the target compounds directly from the effluent. However, as a polyhydroxyl compound, HP-β-CD possesses a good solubility in the aqueous phase while ibuprofen is more soluble in the organic phase. Therefore, in order to ensure the recognition ability of HP-β-CD toward the enantiomers, HP-β-CD was added into the mobile phase (the aqueous phase), in which a head-to-tail elution mode was selected. Meanwhile, the flow rate and revolution speed were set at 1.2 mL/min and 850 rmp, respectively.

The recycling mode was carried out at 70 min immediately before the first peak was about to emerge. As shown in Figure 4, the two enantiomers were co-eluted for their negligible difference in *K*-values. Fortunately, a peak with a shoulder appeared and tended to split into two peaks through one cycle, thus indicating

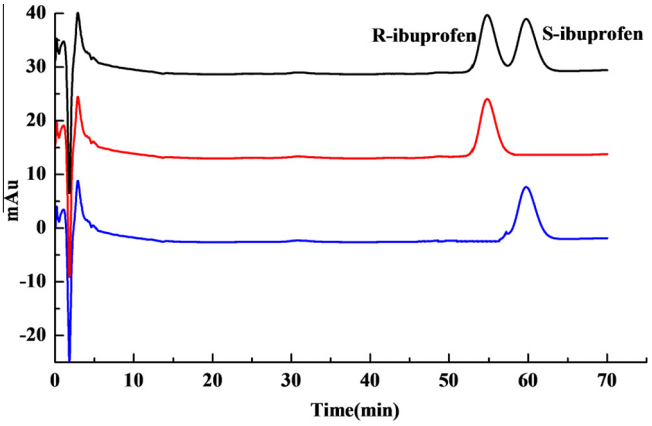

Figure 5. HPLC chromatograms of peak fractions recovered from chiral high-speed counter-current chromatography separation.

that the enantiomers of ibuprofen might potentially be separated by this solvent system. Therefore, another cycle was necessary to further separate the racemates. After an enantioseparation process of 440 min, a relatively enhanced enantioseparation result and S_F of 53.73% were obtained by recycling high-speed counter-current chromatography. Two peak fractions were collected, released and combined.

The purification of the peak fractions was performed in order to remove the chiral selector before HPLC analysis. Figure 5 presents the HPLC chromatogram of pure ibuprofen enantiomers, in which the purities of both enantiomers are over 97.5%. As a result, 13 mg of (*R*)-ibuprofen and 12 mg of (*S*)-ibuprofen were obtained with a recovery of 82% and 89% respectively.

3. Conclusion

An enantioseparation method employing recycling high-speed counter-current chromatography with HP-β-CD as the chiral selector was established to separate (*RS*)-ibuprofen. The two-phase solvent system was composed of *n*-hexane–ethyl acetate–0.1 M phosphate buffer solution with a volume ratio of 7:3:10. The optimal conditions for the recycling high-speed counter-current chromatography operation were at a pH of 2.5, a temperature of 10 °C, and HP-β-CD concentration of 0.1 M in the mobile phase. As a result, a high enantiopurity (over 97.5%) and a recovery rate of 82–89% were achieved. The results demonstrated the feasibility of recycling high-speed counter-current chromatography in the preparative separation of chiral compounds with minor *K*-value differences.

4. Experimental

4.1. Apparatus

A TBE-300B high-speed counter-current chromatography (Tauto Biotechnique Co. Ltd, Shanghai, China) was employed in the preparative enantioseparation. The apparatus is comprised of an upright coil type-J planet centrifuge with three multilayered coils connected in series (diameter of tube, 1.6 mm, total capacity 260 mL) and a 20 mL manual sample loop. The revolution speed can be regulated with a speed controller in the range from 0 to 1000 rpm. The high-speed counter-current chromatography system is composed of a TBP-1002 pump, a TBD-2000 UV detector, a HX-1050 constant temperature regulator (Boyikang Lab Implement Co. Ltd, Beijing, China), and a WH V4.0 workstation (Wuhao Information Technology Co. Ltd, Shanghai, China).

4.2. Reagents

Racemic ibuprofen and β-CD derivative were purchased from J&K chemical Scientific Co., Ltd (Shanghai, China) and Qianhui Fine Chemical & Co. Inc. (Shandong, China), respectively. Methanol and acetonitrile, which were used for HPLC analysis, were of chromatographic grade (Merk, Darmatadt, Germany). *n*-Hexane, cyclohexane and ethyl acetate used for testing *K*-values and high-speed counter-current chromatography separation were of analytical grade and bought from Chemical Reagent Factory of Hunan Normal University (Hunan, China). Ultrapure water (18.2 MΩ resistivity) was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

4.3. Analytical method

The quantification of ibuprofen enantiomers was performed using Dionex Ultimate 3000 HPLC (USA). The analytical column was ANW C18 column (250 mm × 4.6 mm i.d., ANPEL Scientific Instrument Co., Ltd, China). The mobile phase was water–methanol–acetonitrile (78:17:5, v/v/v), containing 100 mM HP-β-CD at a flow rate of 1.0 mL/min. The operating conditions were as follows: detection wavelength, 254 nm; injection volume, 20 μL; column temperature, 25 °C.

4.4. High-speed counter-current chromatography separation

4.4.1. Selection of the solvent system

To secure a suitable two-phase solvent system, HPLC was used to determine *K*-values of various solvent systems including cyclohexane–water, *n*-hexane–ethyl acetate–water, and *n*-hexane–methanol–water with various volume ratios (water phase was replaced by phosphate buffer solution with pH = 2.5 and HP-β-CD concentration of 100 mM). Firstly, an equivalent amount of ibuprofen and β-CD derivative was dissolved in the organic phase and the aqueous phase, respectively. Next, a variety of solvent systems in different volume ratios were prepared and fully mixed (1:1, v/v). The two phase system was shaken vigorously for 3 h before being placed in a water bath at a constant temperature for 3 h. After the extraction equilibrium was reached, the two layers were completely separated and analyzed by HPLC. The *K*-value of a solute was identified according to the following equation: $K = A_U/A_L$, where A_U and A_L indicate the peak areas of solutes in the upper and lower phases respectively.

4.4.2. Preparation of the two-phase solvent system and sample solution

A solvent system consisting of *n*-hexane–ethyl acetate–0.1 M phosphate buffer solution (7:3:10, v/v/v) with pH of 2.5 and HP-β-CD concentration of 0.1 mol/L was used for recycling high-speed counter-current chromatography separation of (*RS*)-ibuprofen. The selected two-phase solvent system was mixed and thoroughly equilibrated in a separatory funnel. Next, they were separated and degassed by ultrasound for 30 min after which a given amount of chiral selector was added in the lower phase. The sample solution for the high-speed counter-current chromatography enantioseparation was prepared by dissolving ibuprofen in 6 mL of the lower phase containing the chiral selector.

4.4.3. High-speed counter-current chromatography separation procedure

Closed recycling high-speed counter-current chromatography was employed to separate the ibuprofen enantiomers. The separation process was conducted under the reversed-phase mode with the upper organic phase as the stationary phase and the lower aqueous phase as the mobile phase. Enantioseparation was initiated by entirely filling the column with the stationary phase. The apparatus was then run at a rotation speed of 850 rpm and the mobile phase was pumped into the column at a flow rate of 1.2 mL/min in the head-to-tail direction. The temperature during the separation process was controlled at 10 °C. Subsequently, the sample solution was injected after the hydrodynamic equilibrium in the column was established by observing the continuous elution of mobile phase at the outlet. Immediately before the target compounds were about to be eluted (R_t = 70 min), the outlet of detector was connected with the inlet of pump to construct a recycling tube. After the enantiomers were separated through several high-speed counter-current chromatography cycles, recycling elution mode was stopped (R_t = 320 min). The effluent from the column was continuously monitored at 254 nm. Finally, the separated enantiomers were released and manually collected according to the chromatogram.

4.5. Recovery of solutes and purity analysis

The peak fractions were collected and acidified before being extracted several times with ethyl acetate. The organic layers were then combined, dried, and evaporated under the reduced pressure to obtain pure enantiomers.

Acknowledgements

This work was financial supported by the National Natural Science Foundation of China (No. 21276282) and the Specialized Research Fund for the Doctoral Program of Higher Education of China (20130162110017).

References

1. Kong, D.; Zhou, Z.; Zhu, H.; Mao, Y.; Guo, Z.; Zhang, W.; Ren, Z. *J. Membr. Sci.* **2016**, *499*, 343–351.
2. Millership, J. S.; Fitzpatrick, A. *Chirality* **1993**, *5*, 573–576.
3. Zhou, J.; Tang, J.; Tang, W. *Trac-Trend. Anal. Chem.* **2015**, *65*, 22–29.
4. Ghanem, A.; Ahmed, M.; Ishii, H.; Ikegami, T. *Talanta* **2015**, *132*, 301–314.
5. Dobrowolski, J. C. *Match-Commun. Math. Co.* **2015**, *73*, 347–374.
6. Fu, Y.; Huang, T.; Chen, B.; Shen, J.; Duan, X.; Zhang, J.; Li, W. *Sep. Purif. Technol.* **2013**, *107*, 11–18.
7. Gourlay, M. D.; Kendrick, J.; Leusen, F. J. *Cryst. Growth Des.* **2008**, *8*, 2899–2905.
8. José, C.; Toledo, M. V.; Briand, L. E. *Crit. Rev. Biotechnol.* **2015**, 1–13.
9. Xun, E.; Wang, Z.; Zhao, J.; Guo, J. *J. Chem. Technol. Biot.* **2015**, *90*, 492–499.
10. Holbach, A.; Godde, J.; Mahendrarajah, R.; Kockmann, N. *AlChE J.* **2015**, *61*, 266–276.
11. Bouquié, R.; Hernando, H.; Deslandes, G.; Daho, A. B. M.; Renaud, C.; Grall-Bronnec, M.; Dailly, E.; Jolliet, P. *Talanta* **2015**, *134*, 373–378.
12. Evans, S. E.; Davies, P.; Lubben, A.; Kasprzyk-Hordern, B. *Anal. Chim. Acta* **2015**.
13. Ito, Y. *J. Chromatogr., A* **2005**, *1065*, 145–168.
14. Zeng, H.; Liu, Q.; Yu, J.; Jiang, X.; Wu, Z.; Wang, M.; Chen, M.; Chen, X. *J. Chromatogr., B* **2015**, *1004*, 10–16.
15. Liu, Q.; Zeng, H.; Jiang, S.; Zhang, L.; Yang, F.; Chen, X.; Yang, H. *Food Chem.* **2015**, *186*, 139–145.
16. Tong, S.; Zheng, Y.; Yan, J. *J. Chromatogr., A* **2013**, *1281*, 79–86.
17. Tong, S.; Yan, J.; Guan, Y.-X.; Lu, Y. *J. Chromatogr., A* **2011**, *1218*, 5602–5608.
18. Tang, K.; Sun, G.; Zhang, P.; Yang, W.; Zhou, C.; Yang, C. *Tetrahedron: Asymmetry* **2015**, *26*, 821–828.
19. Tong, S.; Guan, Y.-X.; Yan, J.; Zheng, B.; Zhao, L. *J. Chromatogr., A* **2011**, *1218*, 5434–5440.
20. Huang, X.-Y.; Di, D.-L. *Trac-Trend. Anal. Chem.* **2015**, *67*, 128–133.
21. Huang, X.-Y.; Ignatova, S.; Hewitson, P.; Di, D.-L. *Trac-Trend. Anal. Chem.* **2015**, *77*, 214–225.
22. Liu, Q.; Xiao, J.; Yu, J.; Xie, Y.; Chen, X.; Yang, H. *J. Chromatogr., A* **2014**, *1363*, 236–241.
23. Liu, Q.; Zhou, J.; Yu, J.; Xie, Y.; Jiang, X.; Yang, H.; Chen, X. *J. Chromatogr., A* **2014**, *1364*, 204–213.
24. Zhang, P.; Sun, G.; Tang, K.; Yang, W.; Sui, G.; Zhou, C. *J. Sep. Sci.* **2014**, *37*, 3443–3450.
25. Zhang, P.; Sun, G.; Tang, K.; Zhou, C.; Yang, C.; Yang, W. *Sep. Purif. Technol.* **2015**, *146*, 276–283.
26. Mucke, L. *Nat. Med.* **2000**, *6*, 973.
27. Long, W. S.; Kow, P. C.; Kamaruddin, A. H.; Bhatia, S. *Process Biochem.* **2005**, *40*, 2417–2425.
28. Adams, S.; Bresloff, P.; Mason, C. *J. Pharm. Pharmacol.* **1976**, *28*, 256–257.

29. Geisslinger, G.; Schuster, O.; Stock, K.-P.; Loew, D.; Bach, G.; Brune, K. *Eur. J. Clin. Pharmacol.* **1990**, *38*, 493–497.
30. Johannsen, M. *J. Chromatogr., A* **2001**, *937*, 135–138.
31. Tian, Y.; Zhong, C.; Fu, E.; Zeng, Z. *J. Chromatogr., A* **2009**, *1216*, 1000–1007.
32. Sajewicz, M.; Piętka, R.; Kowalska, T. *J. Liq. Chromatogr. Related Technol.* **2005**, *28*, 2499–2513.
33. Sajewicz, M.; Piętka, R.; Kowalska, T. *JPC J. Planar. Chromatogr.* **2004**, *17*, 173–176.
34. Peper, S.; Lübbert, M.; Johannsen, M.; Brunner, G. *Sep. Sci. Technol.* **2002**, *37*, 2545–2566.
35. Bonato, P. S.; Del Lama, M. P. F.; de Carvalho, R. *J. Chromatogr., B* **2003**, *796*, 413–420.