

Enantioseparation by Dual-flow Countercurrent Extraction: Its Application to the Enantioseparation of ($\pm$)-Propranolol

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Received February 13, 1996; accepted April 12, 1996

Enantioseparation of ($\pm$)-propranolol has been demonstrated by countercurrent extraction with a two-phase system composed of a chloroform solution of didodecyl L-tartrate (100 mM) and an acetate buffer (50 mM, pH 4.4) containing boric acid (100 mM). The free base of ($\pm$)-propranolol (1.6 g) was dissolved in the aqueous phase and extracted five times by means of dual-flow countercurrent extraction. After an additional five extractions for recovery, the crude *R*-(+)- or *S*-(-)-form was obtained from the aqueous extracts or organic extracts, respectively. They were isolated as their hydrochloride salts with a purity of 89.8% ee (*R*-form, 385.7 mg) and 88.3% ee (*S*-form, 429.5 mg), respectively. They were purified to over 99% ee by recrystallizing twice from 1-propanol.

Key words didodecyl L-tartrate; boric acid; propranolol; enantioseparation; liquid-liquid extraction

Most β -adrenoceptor blocking drugs exist as pairs of optical isomers and almost all the β -blocking activity is found in the (-)-isomers that have 50 to 100 times the inhibitory activity of the (+)-isomers.¹⁾ Because the two stereoisomers of β -blockers can be used to differentiate between the pharmacological effect of β -blockade and other effects, it is important to obtain both enantiomers in pure form.

Tartaric acid and its derivatives have been used for enantioseparation involving chromatographic techniques and extractions. Extractive optical resolution of racemic compounds using (+)-diethyltartrate,²⁾ or (+)-dialkyltartrate in the presence of sodium hexafluorophosphate has been reported.³⁾ We recently investigated the enantioselective distribution behavior of racemic β -blockers in a liquid-liquid two phase system which consisted of a chloroform solution of (+)-didodecyl L-tartrate and an aqueous solution of boric acid.⁴⁾ We also described a dual-flow countercurrent extraction apparatus and its application to the enantioseparation of ($\pm$)-mandelic acid.⁵⁾ Using a solution of L-tartrate in chloroform and an aqueous boric acid solution, we tried to separate ($\pm$)-propranolol by this method. However, this solvent system was unsuitable because of the large difference in the density of the two phases, and also because of the high surface tension of the two liquids. The separation factor obtained with ($\pm$)-propranolol was large enough (to 2.7) to execute the enantioseparation by batch extraction. Thus, in this report, we describe the enantioseparation of ($\pm$)-propranolol in several steps involving dual-flow countercurrent batch extraction.

Experimental

Chemicals Propranolol hydrochloride, boric acid and chloroform were purchased from Wako Pure Chemicals (Osaka). Authentic samples of (*R*)-(+)- and (*S*)-(-)-propranolol hydrochloride were obtained from Aldrich Chemical Co. Inc. (Milwaukee, U.S.A.). (+)-Didodecyl L-tartrate (L-DDT) was prepared as described before.⁴⁾

The free base of ($\pm$)-propranolol was prepared. After mixing an aqueous sodium hydroxide solution (1 M, 110 ml) and ($\pm$)-propranolol hydrochloride (20.0 g, solid) with dichloromethane (250 ml), the two phases were separated using a separating funnel. The aqueous layer was extracted with dichloromethane (100 ml) and the combined organic phase was washed with brine and dried over magnesium sulfate. After filtration,

the filtrate was concentrated under reduced pressure to obtain crude ($\pm$)-propranolol which was recrystallized from dichloromethane and *n*-hexane to yield 10.95 g free base. Another crop of propranolol crystals was obtained from the mother liquor (4.77 g).

Liquid-Liquid Two Phase System The organic phase was a chloroform solution of L-DDT (100 mM). The aqueous phase was prepared by dissolving boric acid (100 mM) in an acetate buffer (50 mM, pH 4.4). Because the change in the volume of the two phases on mixing was negligible, two phases were prepared separately.

Measurement of Distribution Ratios of ($\pm$)-Propranolol Enantiomers The distribution ratios (*D*) of the enantiomers of ($\pm$)-propranolol were measured as described before.⁴⁾ *D* is expressed by:

$$D = \frac{[\text{Prop}]_{\text{org}}}{[\text{Prop}]_{\text{aq}}}$$

Where $[\text{Prop}]_{\text{org}}$ and $[\text{Prop}]_{\text{aq}}$ are the total concentrations of propranolol enantiomers in the organic and aqueous phase, respectively. The measured values of *D* over the concentration range 0.1 to 30 mM are shown in Table 1.

HPLC Analysis For HPLC analysis, a JASCO HPLC system 980 (Japan Spectroscopic Co., Ltd., Tokyo) equipped with a Rheodyne sampling valve (20 μ l) was used. For separation, a Chiralcel OD-R (4.6 i.d. $\times$ 250 mm, Daicel, Japan) column was used and the mobile phase was 0.1 M potassium hexafluorophosphate : acetonitrile = 60 : 40.

Extraction Procedure: Enantioseparation of ($\pm$)-Propranolol by Dual-flow Countercurrent Batch Extraction To a 300 ml separating funnel was added 177 ml of the aqueous phase (*H*) in which 1.6 g of ($\pm$)-propranolol was dissolved and 103 ml of the organic phase (*S*).⁶⁾ The extraction procedure is summarized in Fig. 1. The ($\pm$)-propranolol

Table 1. Distribution Ratios of ($\pm$)-Propranolol Enantiomers

Concentration (mM)	Distribution ratio		
	<i>D_R</i>	<i>D_S</i>	α
0.1	1.11	2.40	2.16
0.5	0.89	2.17	2.60
1.0	0.84	2.16	2.57
2.5	0.82	2.22	2.71
5.0	0.95	2.42	2.55
7.5	0.94	2.46	2.62
15.0	0.99	2.51	2.54
20.0	1.07	2.66	2.49
25.0	1.16	2.73	2.35
30.0	1.36	2.97	2.18

The organic phase is a 100 mM didodecyl L-tartrate chloroform solution and the aqueous phase is an acetate buffer (pH 4.4) containing boric acid (100 mM). The separation factor (α) is calculated by D_S/D_R .

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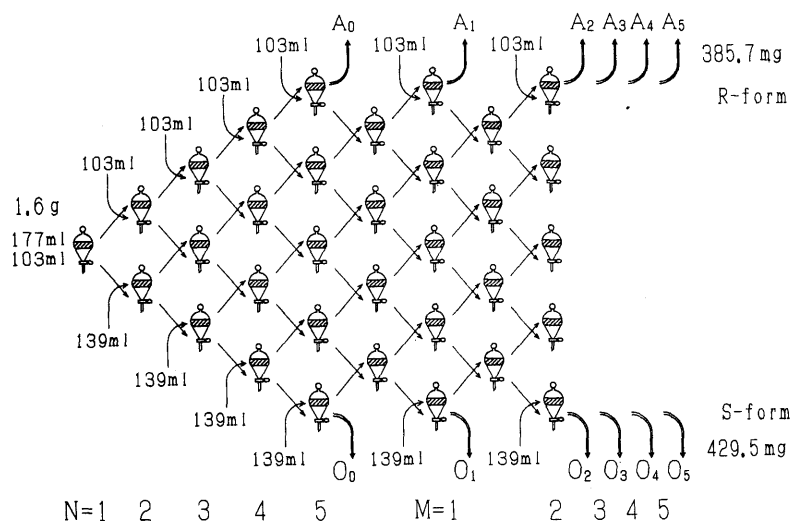
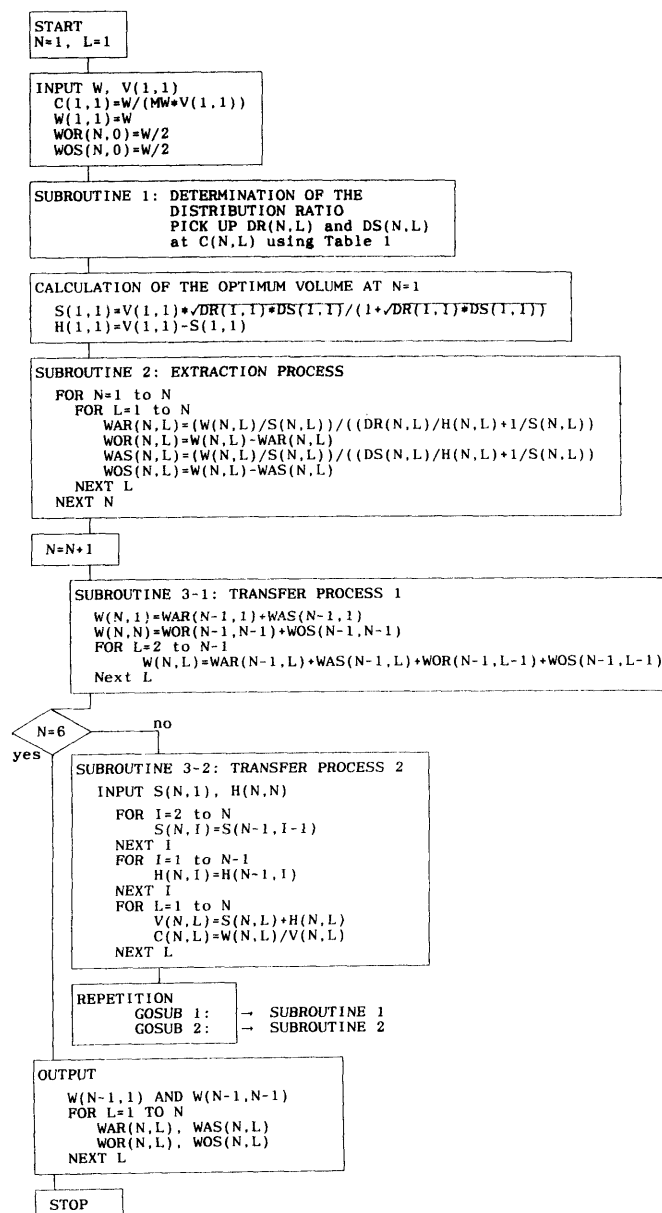


Fig. 1. Dual-flow Countercurrent Batch Extraction Procedure

Five extractions ($N=1$ to 5) with five extractions for recovery ($M=1$ to 5); see text.



aqueous phase was shaken with the organic phase ($N=1$). The organic phase was then transferred to the second separation funnel.

Then, to the aqueous phase in the first funnel was added fresh organic phase (103 ml), and fresh aqueous phase (139 ml) was added to the organic phase in the second funnel and extractions were performed ($N=2$). After another three repetitions of these extraction processes as shown in Fig. 1 ($N=5$), the fifth organic (O_0) and aqueous extracts (A_0) were separated. The remaining phases were mixed in the four funnels as shown in Fig. 1. Then another extraction for recovery ($M=1$) was executed. After an additional four repeats of the recovery procedures ($M=2$ to $M=5$), combined aqueous (A_0 — A_5) and organic extracts (O_0 — O_5) were obtained.

To the combined aqueous extracts, 5.0 g solid sodium hydroxide was added and after dissolving, the solution was extracted with dichloromethane (700 ml). The dichloromethane layer was washed with brine and dried over anhydrous magnesium sulfate. After filtration, the solvent was removed under reduced pressure to yield the crude *R*-form. This was dissolved in 3.0 ml tetrahydrofuran and precipitated as its hydrochloride salt by the addition of 0.3 ml 6 *N* hydrochloric acid. The precipitate was collected and washed with ether (385.7 mg, 89.8% ee by HPLC). After recrystallizing twice from 1-propanol, 174.6 mg (*R*)-(+)-propranolol was obtained with an optical purity of 99.4% ee as shown by HPLC analysis.

The combined organic phases were extracted twice with 1 *M* formic acid (1000 ml, 300 ml). Solid sodium hydroxide (55 g) was added to the acid extracts which were back-extracted twice with dichloromethane (1000 ml, 200 ml). The combined organic layers were washed with brine and dried over anhydrous magnesium sulfate. After filtration and removal of solvent, conversion to the hydrochloride salt gave the crude *S*-form (429.5 mg, 88.3% ee). After recrystallizing twice from 1-propanol, 205.8 mg (*S*)-(–)-propranolol hydrochloride was obtained with an

Fig. 2. Flow Chart of the Calculation Program (for $N=5$)

Calculation process is summarized in the text. The calculation results of $WAR(N,1)$ (=amount of *R*-form in the aqueous phase of the first funnel of the N th stage) and $WOS(N,N)$ (=amount of *S*-form in the organic phase of the N th funnel of the N th stage) are shown in Fig. 3.

Abbreviations: N , number of extraction stage; L , funnel number in each stage (cf. Figs. 1 and 3); W , initial amount of sample (g) = $W(1,1)$; MW , molecular weight of sample (g); $V(N,L)$, total volume of both solvents in the L th funnel of the N th stage; $C(N,L)$, total concentration of both enantiomers in the L th funnel of the N th stage; $DR(N,L)$, distribution ratio of *R* enantiomer at a concentration of $C(N,L)$; $DS(N,L)$, distribution ratio of *S* enantiomer at a concentration of $C(N,L)$; $S(N,L)$, volume of the organic phase in the L th funnel of the N th stage; $H(N,L)$, volume of the aqueous phase in the L th funnel of the N th stage; $W(N,L)$, total amount of both enantiomers in the L th funnel of the N th stage; $WAR(N,L)$, amount of *R*-enantiomer in the aqueous phase in the L th funnel of the N th stage; $WAS(N,L)$, amount of *S*-enantiomer in the aqueous phase in the L th funnel of the N th stage; $WOR(N,L)$, amount of *R*-enantiomer in the organic phase in the L th funnel of the N th stage; $WOS(N,L)$, amount of *S*-enantiomer in the organic phase in the L th funnel of the N th stage.

						N=5, L=1	
						110.6	89.9
						13.2	12.7
				N=4, L=1		152.8	133.2
						31.4	30.1
				N=5, L=2		164.3	149.9
						65.9	64.9
				N=5, L=3		139.5	155.2
						159.1	161.7
				N=5, L=4		63.1	74.1
						171.9	191.1
				N=5, L=5		9.8	13.5
						89.9	80.7
				N=4, L=4		23.7	32.4
						136.6	129.8
				N=3, L=3		62.8	77.4
						202.9	204.4
				N=3, L=2		200.9	208.1
						226.7	230.5
				N=2, L=2		173.7	182.3
						313.8	316.8
				N=2, L=1		318.4	311.6
						176.9	184.3
				N=1, L=1		492.1	486.2
						489.2	488.2
				Exp.			
				Calc.			

Fig. 3. Extractive Separation Process of Propranolol Enantiomers by Dual-flow Countercurrent Batch Extraction

The amount (mg) of *R*(+)-enantiomer in the aqueous phase (Aq, $WAR(N, L)$) and *S*(-)-enantiomer in the organic phase (Org, $WOS(N, L)$) determined in the *L*th funnel of the *N*th stage by experiment (left) and by calculation (right) are shown (cf. Figs. 1 and 2).

optical purity of 99.0% as shown by HPLC analysis.

Calculation Method To calculate the extraction process data, a PC-9801-US microcomputer (Nihon Electric Co., Tokyo) and a program written in N88-BASIC were used. The outline of the program is shown in Fig. 2.

Calculation starts by inputting the initial amount (g) of ($\pm$)-propranolol (*W*) and the total volume of both phases ($V(1, 1)=280$ ml). According to the measured distribution ratios (Table 1), the values of the distribution ratios of the enantiomers in the first plate, $D_R(1, 1)$ and $D_S(1, 1)$ at the initial concentration of $C(1, 1)$ (22 mM) were obtained. Using these values, the optimum volumes of the organic ($S(1, 1)=103$ ml) and aqueous phase ($H(1, 1)=177$ ml) were determined.⁶⁾ Under these conditions, the total amount of both enantiomers in the aqueous phase of $N=1(WA(1, 1))$ and that in the organic phase ($WO(1, 1)$) were calculated. Where, $WA(1, 1)$ represented the sum of the *R*-enantiomer ($WAR(1, 1)$) and *S*-enantiomer ($WAS(1, 1)$) in the aqueous phase, and $WO(1, 1)=WOR(1, 1)+WOS(1, 1)$. After several trial calculations to make the enantio-excess in both extracts become almost equal after five extractions by inputting $S(N, 1)$ and $H(N, N)$ in the subroutine 3-2 of the calculation program (Fig. 2), the volumes of both phases after the second stage were set at $S=103$ ml and $H=139$ ml. Then, the amount of enantiomers in both phases in each funnel from $N=1$ to 5 were calculated by considering the concentration-dependence of the distribution ratios. The calculated results are shown in Fig. 3. The amount of the enantiomers after recovery extractions ($M=1$ to 5) can be calculated similarly.

Results and Discussion

Concentration-Dependence of the Distribution Ratio of ($\pm$)-Propranolol Enantiomers A concentration-dependence of the distribution ratio of the enantiomers was observed (cf. Table 1). This may be mainly because of the pH change of the aqueous phase. At a concentration of 30 mM, for example, the pH of the aqueous phase was changed from 4.4 to 5.0 by the addition of the free base of propranolol. Another reason may be the co-extraction of boric acid with propranolol.⁴⁾ This results in a change in the concentration of boric acid in the aqueous phase, and is not negligible when the concentration of propranolol is relatively high compared with that of the boric acid in the aqueous phase.

Comparison of Calculated and Experimental Data The calculated and experimentally determined amounts of *R*-propranolol enantiomer in the aqueous phase and *S*-enantiomer in the organic phase in each funnel at each stage are summarized in Fig. 3. The difference in the

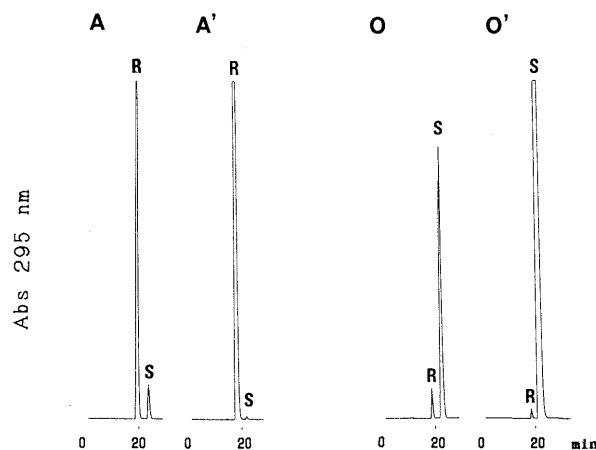


Fig. 4. HPLC Chromatograms of the Enantiomers in the Combined Aqueous Extracts (A, A_0-A_5), in the Organic Extracts (O, O_0-O_5 , cf. Fig. 2), and in the Purified Samples after Two Recrystallizations from 1-Propanol as Their Hydrochloride Salts

A' , 99.4% ee and O' , 99.0% ee.

amounts of enantiomers calculated and found experimentally was considered to be caused by changes in the pH of the aqueous phase and in the concentration of boric acid, as described above. Co-extraction of boric acid with propranolol into the organic phase was not negligible in the first stage where the concentration of propranolol was relatively high (22 mM) compared with that of boric acid (100 mM). When solid boric acid was added to the aqueous phase after the first extraction stage, the distribution of both enantiomers in the second stage was found to be much closer to the calculated values.

Isolation of (+)- and (-)-Propranolol The composition of the enantiomers in the combined aqueous (A_0-A_5) and organic extracts (O_0-O_5), and after two recrystallizations from 1-propanol are shown in Fig. 4 as their hydrochloride salts. Satisfactory results were obtained. Thus, the purity and recovery of the isolated salts of the *R*-form were 99.4% ee and 174.6 mg (21.8%), and those of the *S*-form were 99.0% ee and 205.8 mg (25.7%), respectively.

Enantioselectivity The absolute configuration of the

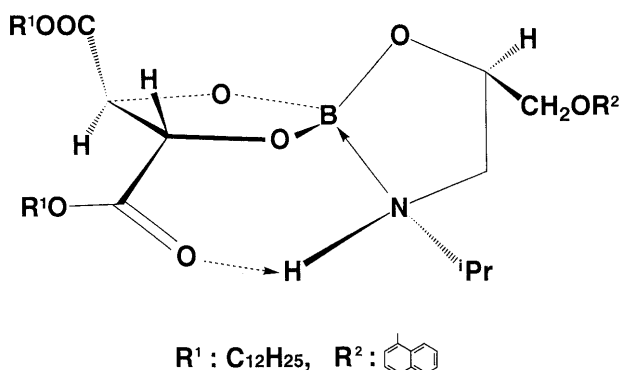


Fig. 5. Proposed Structure of the Three-Component Complex of Didodecyl-L-tartrate-Boric Acid-(*S*)-Propranolol (1:1:1)

There may be hydrogen bonding between the imino group of (*S*)-propranolol and one of the carbonyl groups of the tartrate, where a seven-membered ring could be formed.

propranolol enantiomer extracted into the organic phase with L-DDT and boric acid was found to be the *S*-form by comparison of the retention times in HPLC with authentic specimens. Considering the structure of the borate complex of *S*-propranolol and L-DDT in the organic phase, there appears to be formation of the tetrahedral borate complex with hydrogen bonding (Fig. 5).

However, the relationship between the structure of the chiral diol and the enantioselectivity in the distribution of racemic amino-alcohols in the liquid-liquid two-phase system in the presence of boric acid requires further

investigation.

Conclusion

In conclusion, enantioseparation by dual-flow counter-current extraction using borate complex formation with didodecyl-L-tartrate for ($\pm$)-propranolol has been demonstrated. A resolution of 1.6 g of ($\pm$)-propranolol provided 385.7 mg of the hydrochloride salt of the (*R*)-(+)-form and 429.5 mg of the (*S*)-(–)-form with the purity of over 85% ee. Purification to over 99% ee was achieved by recrystallization from 1-propanol.

References and Notes

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- 6) After setting the total volume as 280 ml, the volume of the aqueous phase was set as $H = 177$ ml, and that of the organic phase was set as $S = 103$ ml by the calculation with the following equations⁷⁾ with using the values of the distribution ratios ($D_R(1,1) = 1.11$ and $D_S(1,1) = 2.69$) at the initial concentration of 22 mM (Table 1).

$$H/S = 1/\sqrt{D_R \cdot D_S}, \text{ and}$$

$$H + S = 280$$
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