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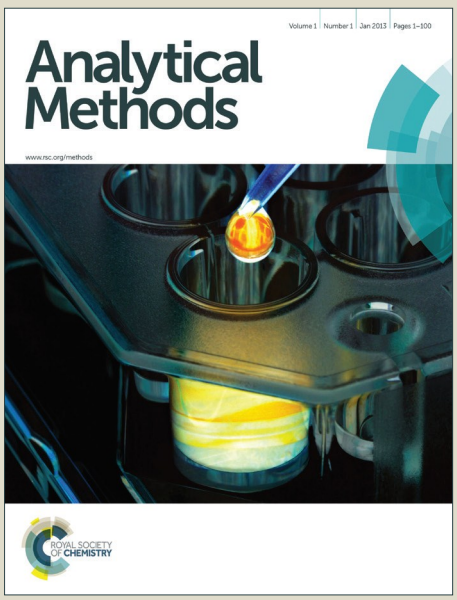

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Enantioseparations of fourteen amino alcohols by nonaqueous capillary electrophoresis using the lactobionic acid/D-(+)-xylose–boric acid complexes as chiral selectors

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Abstract: An interesting study on chiral nonaqueous capillary electrophoresis (NACE) was developed in this paper. Two new chiral selectors, lactobionic acid–boric acid complex and D-(+)-xylose–boric acid complex were respectively *in situ* synthesized in nonaqueous background electrolytes (BGEs) containing methanol and triethylamine. They were found to be applicable for the enantioseparations of fourteen amino alcohols including eight β-blockers and six β-agonists by NACE. In order to achieve good enantioseparations, the effects of chiral selector concentration, BGE composition, capillary temperature, and applied voltage were systematically investigated. Under the optimized conditions, most of the tested amino alcohols achieved good chiral resolutions. The effects of the molecular structures of chiral selectors and analytes on enantioseparations were discussed in terms of molecular interactions. The method was proved to be suitable for routine analysis of propranolol enantiomers, since it provided satisfactory results during linearity, precision, and

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accuracy (recovery) studies using lactobionic acid–boric acid complex as chiral selector. Good linearity was obtained in a range of 1.0 ~ 100.0 μg mL^{-1} for each propranolol enantiomer and the recoveries for them were ranged from 96.4% to 105.9% with the relative standard deviation (RSD) less than 6.4%. This chiral NACE method was applied for the determination of propranolol enantiomers from tablets and the average contents were 101.3% for (*R*)-propranolol and 98.5% for (*S*)-propranolol (n = 5).

1. Introduction

Amino alcohols such as β-agonists and β-blockers are chiral hydroxyl-amine-containing compounds. Sympathomimetic drugs, for instance, clenbuterol, bambuterol and terbutaline with potent β_2-adrenoceptor stimulating properties are commonly used in the treatment of respiratory diseases. These drugs are usually administered as racemic mixtures, while the desired pharmacologic effect is largely dependent on their (*R*)-enantiomer.[1] For instance, the (*R*)-enantiomer of clenbuterol is a stimulant for β_2-receptors while the (*S*)-enantiomer reveals a blocking effect on β_1-receptors.[2] Therefore, it is of great importance to develop simple and effective analytical methods for their enantiomeric resolutions.

During the past decades, CE has shown to be an attractive and powerful separation technique in chiral drug discovery and manufacturing processes. It could provide high separation performace, high sensitivity, short analysis time, and low sample and chiral selector consumption.[3-8] Nowadays, nonaqueous capillary electrophoresis (NACE) is recognized as a good alternative to aqueous CE for chiral

analysis because of many advantages.[9] It facilitates the use of chiral selectors with a low solubility in water and enables non or poorly watersoluble substances to be analyzed.[3,10-12] It can improve the enantioselectivity of chiral selector by choosing a suitable organic solvent or mixture of organic solvents. Changing the composition of background electrolyte (BGE) can also significantly affect the migration time and separation performance. In addition, lower conductivity of electrolytes in NACE leads to less Joule heat, allowing higher voltage to apply and higher buffer concentration to use.[13] Furthermore, the special physicochemical properties of the nonaqueous solvents facilitate the hyphenation to mass spectrometer (MS).[14]

The most common chiral selectors used in NACE are cyclodextrins (CDs),[15-18] macrocyclic antibiotics,[19,20] chiral ion-pair complexes,[21-24] and so on. In our previous work, a series of D/L-tartrate–boric acid complexes and two polyols–boric acid complexes were *in situ* synthesized and used as chiral ion-pair selectors in NACE for the enantioseparations of some amino alcohols.[22-24] However, the developed methods were not applied to the analysis of real samples. In this paper, two chiral selectors, lactobionic acid–boric acid complex and D-(+)-xylose–boric acid complex were also respectively *in situ* synthesized in nonaqueous BGEs containing methanol and triethylamine. They were firstly adapted and employed in NACE. One aim of this work wasis to test the chiral separation performance of the two polyhydroxy compounds–boric acid complexes for the eight β-blockers and six β-agonists by NACE. The effects of the molecular structures of chiral selectors and analytes on enantioseparations would be also discussed. The other aim was to validate the

analytical performance and feasibility of this chiral NACE method for the determination of enantiomers in real samples.

2. Experimental

2.1 Instrumentation

NACE experiments were conducted on a TH-3100 high performance capillary electrophoresis system (Tianhui Institute of Separation Science, Baoding, China) equipped with a thermostatic system and a UV detector. Data were collected with a CXTH-3000 chromatography workstation. All NACE separations were carried out in a 50 μm I.D. (Yongnian Reafine Chromatography Co., Ltd., Hebei, China) uncoated fused silica capillary with a total length (L_{tot}) of 55.0 cm and an effective length (L_{eff}) of 45.0 cm. The new capillary was conditioned by flushing with methanol for 10 min, 1.0 M NaOH solution for 20 min, distilled water for 5 min, 1.0 M hydrochloric acid solution for 20 min and distilled water for 5 min in sequence. Before each run the capillary was rinsed with running buffer for 3 min. All chiral analytes were detected at 214 nm. Samples were introduced using positive pressure injection at 2.9 psi for 2 s.

2.2 Chemicals and materials

Racemic propranolol hydrochloride, metoprolol tartrate, esmolol hydrochloride, bisoprolol fumarate, sotalol hydrochloride, atenolol, cycloclenbuterol hydrochloride, clenbuterol hydrochloride, bambuterol hydrochloride, tulobuterol hydrochloride and clorprenaline hydrochloride were purchased from the National Institute for Food and Drug Control (NIFDC, Beijing, China). The following racemic compounds were extracted by methanol from medicine tablets: carvedilol (JUNEN®, Beijing Juneng

Pharmaceuticals Co., Ltd., China), propafenone (LUMING®, Shandong Renhetang Pharmaceuticals Co., Ltd., China), and terbutaline sulphate (BRICANYL®, AstraZeneca Pharmaceuticals Co., Ltd., China). The commercially available tablets of propranolol hydrochloride were purchased from Tianjin Lisheng Pharmaceuticals Co., Ltd. (Tianjin, China). (*R*)-propranolol hydrochloride and (*S*)-propranolol hydrochloride werewas purchased from Sigma–Aldrich (St. Louis, MO, USA).

Lactobionic acid (purity ≥ 97 wt%) and D-(+)-xylose(purity ≥ 98 wt%) were purchased from Aladdin (Shanghai, China). Boric acid was the product of Baoding Chemical Reagent Factory (Baoding, China). Triethylamine (water content ≤ 0.2 wt%) was supplied by Tianjin Kermel Chemical Reagent Co., Ltd. (Tianjin, China). Methanol, chromatographic reagent grade, was purchased from Tianjin Concord Technology Co., Ltd. (Tianjin, China). Ultra-pure water was used to prepare all of the solutions. The other reagents and chemicals were all of analytical reagent grade and used as received.

2.3 Preparation of buffer and sample solutions

NACE running buffers were prepared daily by weighing the desired quantities of lactobionic acid/D-(+)-xylose, boric acid, and dissolving them in methanol to the desired volume in a flask. The appropriate concentrations of triethylamine were added into running buffers to control their apparent pH (pH*) values.

All of the racemic samples used for NACE chiral separation were dissolved in methanol to make stock solutions of 0.5 mg/mL 500.0 μg mL^{-1} and diluted with methanol to 25.0 μg mL^{-1} (for propranolol, clenbuterol and cycloclenbuterol) or 50.0

μg mL^{-1} (for the other analytes). All of the stock solutions of stardard samples used for method validation were prepared by dissolving proper quantities of (*R*)-propranolol or (*S*)-propranolol in methanol to make a concentration of 1.0 mg mL^{-1}. The solutions were stored at 4 ℃ and brought to ambient temperature each time before use. All of the solutions were filtered through a 0.22 μm syringe type filter prior to use.

2.4 Calculations

The main performance parameter in this work is a resolution (*Rs*). It is calculated according to $Rs = 2(t_2 - t_1) / (w_1 + w_2)$, where t_1 and t_2 are the migration times of the two enantiomers, and w_1 *and* w_2 are the the widths of their peaks at the baseline.[22]

3. Results and discussion

3.1 *In situ* synthesis of lactobionic acid–boric acid complex and D-(+)-xylose–boric acid complex chiral selectors

In this study, interesting phenomena were found that chiral analytes could not be resolved with the BGEs containing only lactobionic acid or D-(+)-xylose without boric acid. This indicated that the real chiral selectors for NACE enantioseparation were not lactobionic acid or D-(+)-xylose itself, but complexes between lactobionic acid/D-(+)-xylose and boric acid, *i.e.*, lactobionic acid/D-(+)-xylose–boric acid complexes. They were *in situ* produced by the reaction of lactobionic acid or D-(+)-xylose with boric acid in buffers. Optical lactobionic acid and D-(+)-xylose are chiral polyols, having *cis*-vicinal or *cis*-interval hydroxyl groups in their molecular

structures (Fig. 1). They are very likely to react with boric acid to produce complex acids, *i.e.* the real chiral selectors resulting in the enantioseparation in NACE. The formation of the complexes limited the rotation of C-C single bond between the two chiral carbon atoms and fixed the chiral centers of lactobionic acid or D-(+)-xylose. Thus, the difference in steric matching capability between the chiral centers of chiral selector and two enantiomers were enhanced. The chiral recognition capability of the complex chiral selectors was significantly improved.[25]

3.2 Optimization of separation conditions using lactobionic acid–boric acid complex as chiral selector

3.2.1 Effects of lactobionic acid and boric acid concentrations on enantioseparation

It is well known that chiral selector plays an important role in the chiral separation. Thus, the concentration of the chiral selector will definitely affect the results of the enantioseparation. In this study, the effects of lactobionic acid and boric acid concentrations in the range of 0 ~ 8 mM and 0 ~ 120 mM were respectively studied with 14.4 mM triethylamine in methanol. The results showed that the *R*s of most of the chiral analytes increased with the increase of lactobionic acid and boric acid concentrations in the buffer. This could be interpreted that the increase in the concentration of either of them would promote the reaction. Thus, the formation of chiral selector would increase, producing the improvement of chiral resolution. As shown in Fig. 2, when the concentration of lactobionic acid increased from 0 ~ 8 mM, the *R*s gradually increased. As higher concentration led to the supersaturation of

lactobionic acid in methanol, 8 mM lactobionic acid was finally selected. The effect of boric acid concentration on the enantioseparation was investigated from 0 ~ 120 mM. As shown in Fig. 3, for most of the chiral analytes, with the boric acid concentration increased from 0 ~ 100 mM, the *R*s gradually increased. When the boric acid concentration increased from 100 ~ 120 mM, the *R*s of most of the chiral analytes increased slightly. Finally, 100 mM boric acid was selected to perform further investigations.

3.2.2 Effect of the BGE pH* on enantioseparation

The pH* of the running buffer is always a very important parameter in NACE. Since the complex reaction of the lactobionic acid and boric acid was reversible, and the complex acid was an acidic protolyte, adding some alkaline electrolyte to the BGE could promote this reaction to produce more chiral selector. This process was beneficial to enantioseparation. In addition, pH* could also affect the degree of the ionization of chiral analytes, which was also very important in chiral separation.[25,26] In this study, triethylamine was used to control the pH* of the BGE. It was added along with the chiral selector. The effect of triethylamine concentration on enantioseparation was investigated from 0 ~ 21.6 mM. The variety of *R*s was shown in 45. It was found that no separation could be achieved when the BGE containing chiral selector without triethylamine. When the triethylamine concentration increased from 0 ~ 14.4 mM, both of the migration times and chiral resolution increased. When the concentration of triethylamine increased to 21.6 mM, the migration times continuously increased, but the resolutions had no obvious change or even decreased. Therefore, 14.4 mM

triethylamine was finally selected.

3.2.3 Effects of capillary temperature and applied voltage on enantioseparation

Changes in capillary temperature can cause variations in efficiency, viscosity, migration times and injection volumes. The effect of capillary temperature on the enantioseparation was investigated in the range of 20 ~ 30 ℃ (steps of 5 ℃). In theory, when the capillary temperature increases, the BGE viscosity will decrease, thus results in the decrease of migration time and resolution. In this study, as the capillary temperature had little effect on the chiral resolution, 25 ℃ was finally selected.

In NACE, high voltage is usually reduces the analysis time. Therefore, the effects of applied voltage on resolution and migration times were studied in the range of 10 ~ 20 kV (steps of 5 kV). Increasing the voltage resulted in shorter migration times, but generation of Joule heat affected the resolution and efficiency. Taking all above into account, 15 kV was selected as the applied voltage as a compromise between the analysis time and baseline appearance.

The optimized NACE conditions are concluded as follows: 8 mM lactobionic acid, 100 mM boric acid, and 14.4 mM triethylamine in methanol; positive pressure injection at 2.9 psi for 2 s; applied voltage, 15 kV; capillary temperature, 25 ℃; detection wavelength, 214 nm. The molecular structures of the fourteen amino alcohols and their enantioseparations under the optimized conditions using lactobionic acid–boric acid complex as the chiral selector are shown in Fig. 5.

3.3 Optimization of separation conditions using D-(+)-xylose−boric acid complex as chiral selector

D-(+)-xylose−boric acid complex was also *in situ* synthesized and applied as a chiral selector for enantioseparations of the fourteen amino alcohols. The optimization of separation conditions and the theory were same to lactobionic acid–boric acid complex. The optimized NACE separation conditions are: 40 mM D-(+)-xylose, 100 mM boric acid, 78.9 mM triethylamine, an applied voltage of 15 kV and a capillary temperature at 25 ℃. As can be seen in Fig. 6, most of the fourteen amino alcohols achieved good chiral resolutions.

3.4 Migration orders of two enantiomers

The identity of the peaks of propranolol enantiomers was determined in two experiments by spiking a single pure (*S*)propranolol into the solution of its racemate. As shown in Fig. 5 and Fig 6, the (*S*) propranolol migrates later than the (*R*)-propranolol. The migration mechanisms have not been presented and further study will continue in the following research. Due to the lack of optical pure standard materials, the migration orders of other enantiomers have not been determined.

3.5 Effects of molecular structures of chiral selectors and chiral analytes on enantioseparation

Similar to our previous work, lactobionic acid and D-(+)-xylose are polyhydroxy compounds which have *cis*-vicinal or *cis*-interval hydroxyl groups in their molecular structures (shown in Fig.1). As we expected, they could also react with boric acid to produce complex acids easily, so both of them obtained good chiral separations under the optimized conditions.

It is well known that the different chiral separation can be resulted from the

different molecular interactions between chiral selector and enantiomers.[25,27] Since saccharides are chiral polyols, having a lot of stereogenic centers and functional groups, so they may have multiple interactions with chiral analytes. Many interactions including electrostatic, hydrogen bonding, steric hindrance, dipole-dipole, hydrophobic interactions and so on are thought to cause chiral recognition. As shown in Fig. 5 and Fig. 6, lactobionic acid–boric acid complex chiral selector obtained better chiral resolutions than D-(+)-xylose−boric acid complex. Maybe this was because lactobionic acid had two pairs of *cis*-vicinal hydroxyl groups in its molecular structure, and either of them might react with boric acid to produce complex chiral selector. Meanwhile, lactobionic acid was an acidic oligosaccharide, electrostatic interaction and hydrogen bonding should be mainly responsible for the enantioseparation with chiral recognition mechanism of ion-pair principle.[28] As shown in Fig. 6, most of the enantioseparations forof β-blockers wereis better than the β-agonists. This might be because these β-blockers (propranolol, metoprolol, esmolol, bisoprolol, atenolol, and propafenone) had a methyleneoxy group between the aromatic portion and the chiral center. Probably, because the interactions of this group with current chiral selector had some favorable effects on chiral recognition, they obtained better enantioseparations than β-agonists. Carvedilol had an aromatic ring connecting to the amino group and obtained a relatively poor enantioseparation.; It was probably that its steric hindrance effect also played an important role in chiral recognition. Certainly, these hypothesis need to be corroborated by other analytical techniques in the further work.

3.6 Validation of the NACE method

The feasibility of the developed method in the analysis of real samples was tested for the determination of propranolol enantiomers from tablets using lactobionic acid–boric acid complex as chiral selector under the above optimized conditions. The analytical performances were evaluated in terms of linearity, limit of determination, limit of quantification, precision, and accuracy (recovery).

Calibration curves were constructed using the areas of the chromatographic peaks measured at seven increasing concentrations, in a range of 1.0 ~ 100.0 μg mL^{-1} for each enantiomer of propranolol. Good linearity was obtained for the two enantiomers with the correlation coefficient (r^2) 0.9996 and the slope and intercept were shown in Table 1.

Based on a signal-to-noise ratio of 3 and 10, the limit of determination (LOD) and limit of quantification (LOQ) of (*R*)-propranolol were 0.25 μg mL^{-1} and 1.0 μg mL^{-1}, and for (*S*)-propranolol were 0.5 μg mL^{-1} and 2.0 μg mL^{-1}, respectively (Table 1).

Precision is a measure of the ability of the method to generate reproducible results. The precision of this method was evaluated using intra-day and inter-day precisions. They were determined at the concentration of 12.5 μg mL^{-1} for each enantiomer in standard solutions. The intra-day and inter-day precisions of the method were expressed as the relative standard deviation (RSD) of analyzing six replicates of standard samples prepared in the same day and three replicates in five different days. The results showed the RSD of the two enantiomers were less than 1.5% for migration

times and less than 2.6% for peak areas (Table 1).

Accuracy experiments were measured by spiking three different concentrations (10.0 μg mL^{-1}, 12.5 μg mL^{-1} and 15.0 μg mL^{-1}) of standard samples of (*R*)- and (*S*)-propranolol into the tablet sample matrix. The recoveries for them were in a range of 96.4% ~ 105.9% with RSD less than 6.4%, which indicated that the method was reliable (Table 1).

The validated method was applied to the determination of propranolol enantiomers from commercially available tablets. The amounts of (*R*)- and (*S*)-propranolol in commercial tablets were calculated using calibration curve method. The average contents of tablets were 101.3% for (*R*)-propranolol and 98.5% for (*S*)-propranolol (n = 5). The results of the assay indicated that the method was selective for the analysis of propranolol and there was no interference from the drug formulation excipients.

4. Conclusions

In this paper, two novel chiral selectors, lactobionic acid−boric acid complex and D-(+)-xylose−boric acid complex, were respectively *in situ* synthesized and applied firstly to separate fourteen chiral amino alcohols by NACE. Good enantioseparations were obtained under the optimized conditions. Effects of the molecular structures of chiral selectors and analytes on enantioseparations were discussed in terms of molecular interactions. The analytical performances, including linearity, precision, and accuracy were also studied and the method was applied for the determination of propranolol enantiomers from commercial tablets. This study

provided a new method for the quality control of some chiral amino alcohol drugs. It was a contribution to the development and application of new chiral selectors of polyhydroxy compounds–boric acid complexes.

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Figure captions

Fig. 1 Structural formulae of lactobionic acid and D-(+)-xylose.

Fig. 2 Effect of lactobionic acid concentration on resolution.

Running buffer composition in addition to the concentration of lactobionic acid is 100 mM boric acid and 14.4 mM triethylamine in methanol. NACE conditions: capillary dimensions, L_{tot} 55.0 cm, L_{eff} 45.0 cm, I.D. 50 μm; positive pressure injection at 2.9 psi for 2 s; applied voltage, 15 kV; capillary temperature, 25 ℃; detection wavelength, 214 nm.

Fig. 3 Effect of boric acid concentration on resolution.

Running buffer composition in addition to the concentration of boric acid is 8 mM lactobionic acid and 14.4 mM triethylamine in methanol. The other conditions are the same as in Fig. 2.

Fig. 4 Effect of triethylamine concentration on resolution.

Running buffer composition in addition to the concentration of triethylamine is 8 mM lactobionic acid and 100 mM boric acid in methanol. The other conditions are the same as in Fig. 2.

Fig. 5 Electropherograms of enantioseparations of analytes under the optimized conditions with lactobionic acid–boric acid complex as the chiral selector.

Running buffer composition: 8 mM lactobionic acid, 100 mM boric acid, and 14.4 mM triethylamine in methanol. The other conditions are the same as in Fig. 2.

Fig. 6 Electropherograms of enantioseparations of analytes under the optimized conditions with D-(+)-xylose–boric acid complex as the chiral selector.

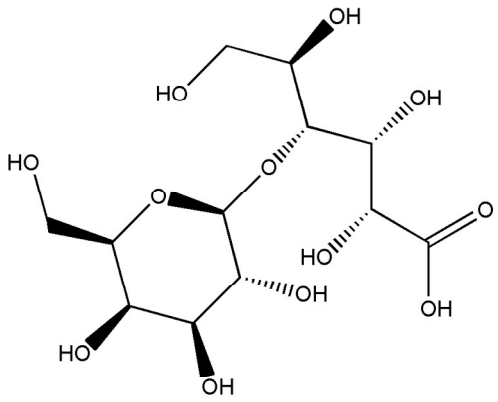
Running buffer composition: 40 mM D-(+)-xylose, 100 mM boric acid, and 78.9 mM triethylamine in methanol. The other conditions are the same as in Fig. 2.

Table 1 The validation results of the NACE method using lactobionic acid–boric acid complex as chiral selector in NACE [a].

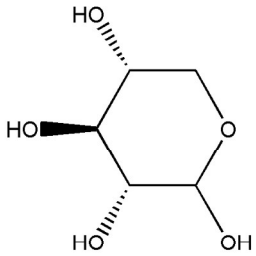
	(*R*)- propranolol	(*S*)- propranolol
Migration time intraday RSD (%) (n = 6)	0.4	0.4
Migration time interday RSD (%) (n = 15)	1.2	1.5
Peak area intraday RSD (%) (n = 6)	1.1	0.6
Peak area interday RSD (%) (n = 15)	2.6	2.3
Slope	4792.7	5317.9
Intercept	-360.83	-1342.4
Correlation coefficient	0.9996	0.9996
LOD (μg mL^{-1})	0.25	0.5
LOQ (μg mL^{-1})	1.0	2.0

	Base value (μg mL^{-1})	Quantity added (μg mL^{-1})	Quantity found (μg mL^{-1})	Recovery (%)	RSD (%)
	11.3	10.0	20.9	96.4	6.4
(*R*)-propranolol	11.3	12.5	24.0	101.8	3.1
	11.3	15.0	26.7	102.6	1.2
	11.0	10.0	20.9	99.2	5.3
(*S*)-propranolol	11.0	12.5	24.2	105.3	3.0
	11.0	15.0	26.9	105.9	0.6

[a] NACE conditions are the same as in Fig. 2.



lactobionic acid



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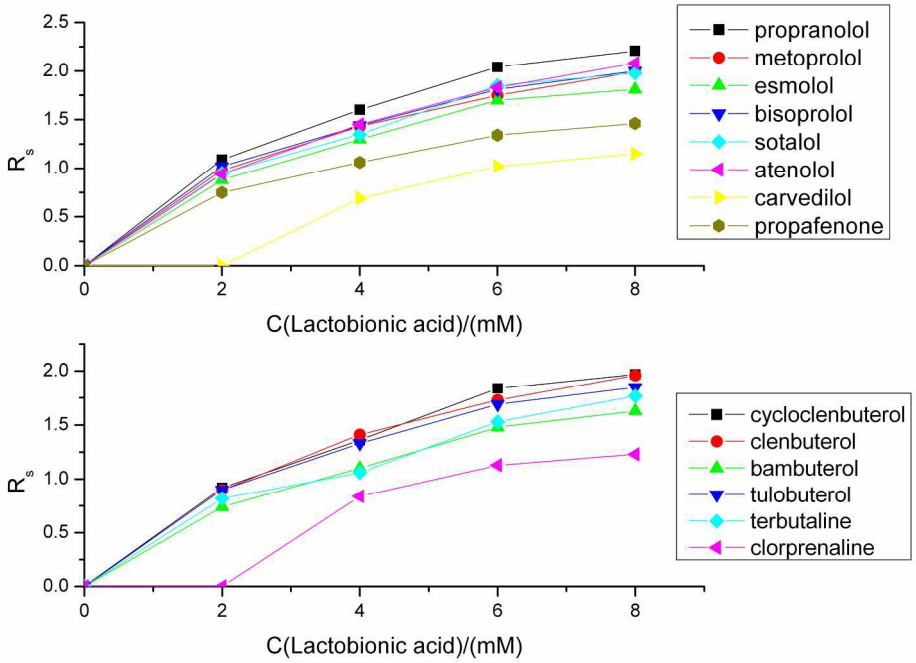

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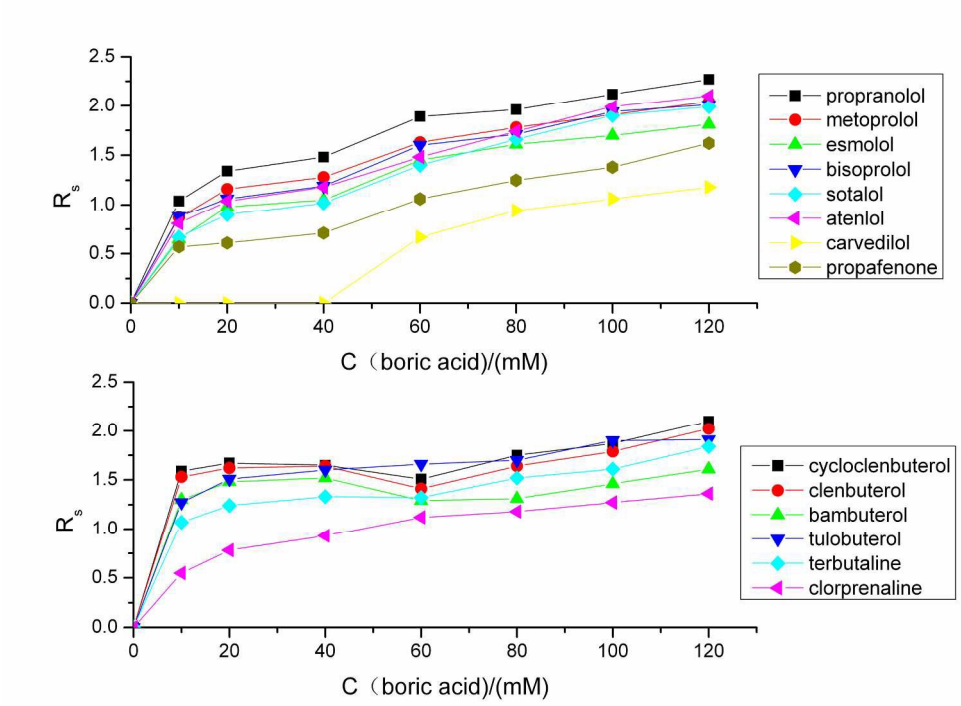
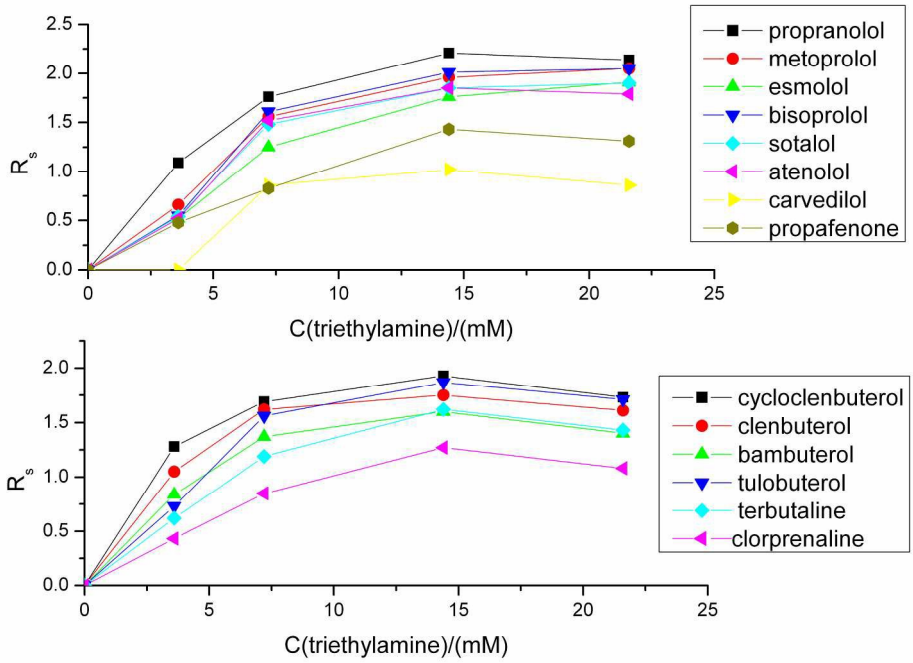
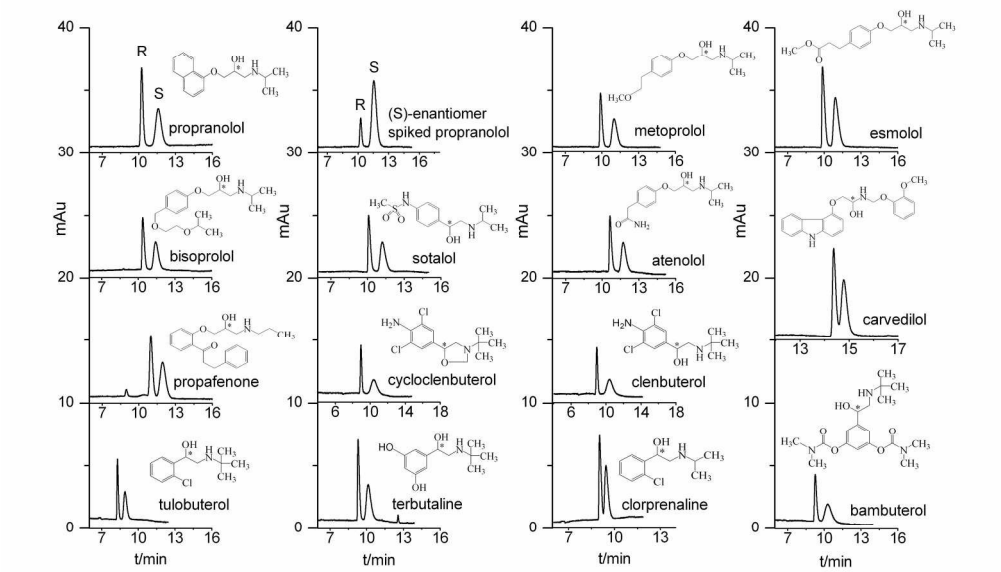


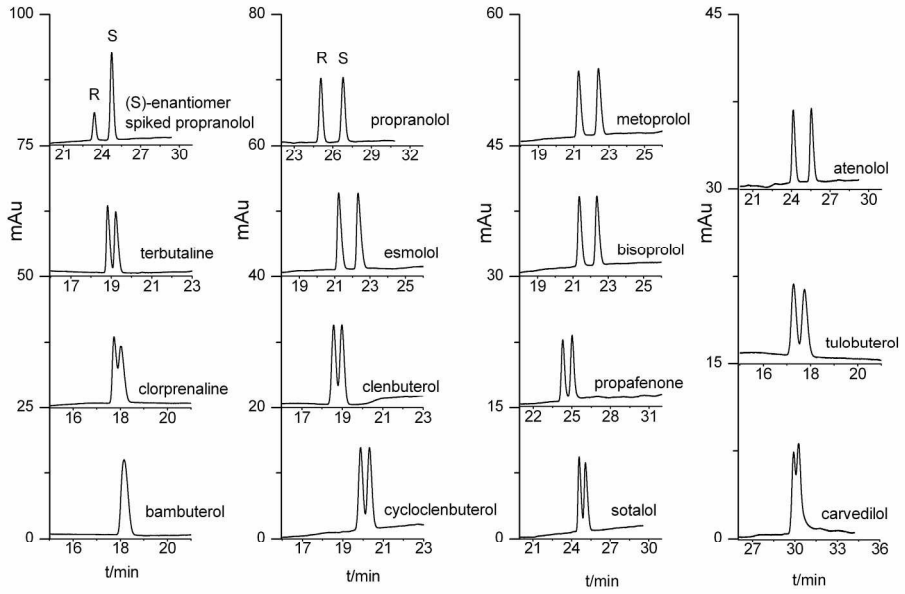
230x173mm (300 x 300 DPI)



230x172mm (300 x 300 DPI)



202x132mm (300 x 300 DPI)



202x138mm (300 x 300 DPI)