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Supplementary Information

Effectively enhancing the enantioseparation ability of β-cyclodextrin derivatives by *de novo* design and molecular modeling

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The effect of BGE pH, HDHSA-β-CD concentration, buffer concentration and voltage on enantiomeric separation of the studied analytes is summarized in below content. The corresponding electropherograms and chemical structures of the chiral drugs separated by CE can be seen in Figures S1-4. The MEP of host/guest complexes are shown in Figure S5. ^{1}H NMR spectrum of HDHSA-β-CD is demonstrated in Figure S6.

Effect of BGE pH

The effect of buffer pH on enantiomeric separation of the studied analytes was investigated over the range of 2.5 to 4.0 using 60.0 mmol L^{-1} phosphate buffer with 10.0 mmol L^{-1} HDHSA-β-CD under 20 kV. Under such conditions, the analytes were completely protonated, thus moving towards the cathode due to the applied electric field. HDHSA-β-CD was negatively charged as a salt of strong acid (pKa = 1.0).

Seen from Table S1, the analytes reached their maximum migration time with the maximum chiral selectively and resolution at pH 4.0, however, only Clorprenaline reached completely chiral separation (Rs > 1.5). Nevertheless, BGE pH has slight effect on the enantiomeric separation (chiral resolution and selective factor). Thence, BGE pH is not the prominent factor. Moreover, it is has been reported that in order to guarantee the analytes

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were completely protonated, the lowest BGE pH is 2.5. Based on the above results, subsequent studies were carried out at pH 2.5.

Table S1. The effect of pH on the t_1(min), R and α of four analytes.

pH	2.5			3.0			3.5			4.0		
	t_1	R	α	t_1	R	α	t_1	R	α	t_1	R	α
Clenbuterol	5.47	0.93	1.012	5.78	0.99	1.015	5.91	1.08	1.016	5.97	1.14	1.016
Tulobuterol	5.40	0.60	1.010	5.63	10.75	1.011	5.73	0.78	1.010	5.81	0.82	1.013
Clorprenaline	5.25	1.50	1.020	4.56	1.61	1.043	4.86	1.56	1.021	4.98	1.55	1.020
Terbutaline	5.97	1.31	1.017	6.13	1.31	1.018	6.27	1.44	1.020	6.41	1.46	1.018

BGE: 10.0 mmol L^{-1} HDHSA-β-CD, 60.0 mmol L^{-1} phosphate buffer, 20 kV;
The enantiosepartion parameters of CE were calculated as follows:
The resolution, R, for a pair of enantiomers was calculated from $R = 2(t_2-t_1)/(W_2+W_1)$; the electrophoretic selectively, α, was calculated as $\alpha = t_2/t_1$; where $W1$ and $W2$ are the peak widths at baseline of each enantiomer and t_1 and t_2 are the migration times of enantiomers 1 and 2.

Effect of HDHSA-β-CD concentration

The effect of the HDHSA-β-CD concentration on the enantioseparation of four basic drugs was examined by gradually increasing the concentration from 5.0 to 20.0 mmol L^{-1} using the optimum pH value for each analyte. As shown in Table S2, with increasing the concentration of CD, the migration times of analytes were generally prolonged. This increase in migration times was partially caused by the increased involvement of the analytes in complex with CD. Meanwhile, the increased ionic strength also contributed for the decrease of effective mobility of analytes. The net result led to longer migration time of analytes with increasing CD concentration.

The chiral resolutions of studied drugs were also dependent on HDHSA-β-CD concentration. Tulobuterol had its maximum resolution values at HDHSA-β-CD concentration of 15.0 mmol L^{-1}. On further in creasing HDHSA-β-CD concentration from 15.0 mmol L^{-1}, the peak width broadened and the *Rs* began to decrease. While for the other analytes the maximum resolution values were achieved at much higher concentrations, indicating that the enantiomers of Tulobuterol had higher affinity for HDHSA-β-CD than that of the latter analytes. Clorprenaline and Terbutaline had reached completely chiral separation at CD concentration of 10.0 mmol L^{-1} and 15.0 mmol L^{-1}, respectively. Based on the above results, the optimal concentrations of HDHSA-β-CD were set at 10.0 mmol L^{-1} for further study.

Table S2. The effect of the concentration of HDHSA-β-CD on the t_1(min), R and α of four analytes.

C	5.0			10.0			15.0			20.0		
(mmol L^{-1})	t_1	R	α	t_1	R	α	t_1	R	α	t_1	R	α
Clenbuterol	5.23	0.69	1.009	5.47	0.93	1.012	5.64	1.03	1.014	5.94	1.14	1.016
Tulobuterol	5.05	0.70	1.011	5.40	0.60	1.010	5.70	0.89	1.013	5.90	0.83	1.013
Clorprenalin e	5.20	1.24	1.016	5.25	1.50	1.020	5.41	1.56	1.023	5.82	1.72	1.026
Terbutaline	5.79	1.09	1.013	5.97	1.31	1.017	6.05	1.36	1.018	6.18	1.50	1.021

BGE: pH 2.5, 60.0 mmol L^{-1} phosphate buffer, 20 kV;
The calculations of enantioseparation parameters of CE are the same as in Table S1.

Effect of buffer concentration

On the basis of preliminary experiments, 40.0, 60.0 and 80.0 mmol L^{-1} sodium phosphate buffer were applied for the optimization. When the buffer concentration increased from 40.0 to 80.0 mmol L^{-1}, the migration times and resolutions of all analytes increased insignificantly. When the buffer concentration was higher than 60.0 mmol L^{-1}, baseline drift became serious. Therefore, 60.0 mmol L^{-1} phosphate buffer was used as the optimum concentration.

Table S3. The effect of the concentration of phosphate buffer on the t_1(min), R and α of four analytes.

H_3PO_4concentration	40.0			60.0			80.0		
(mmol L^{-1})	t_1	R	α	t_1	R	α	t_1	R	α
Clenbuterol	5.26	0.90	1.010	5.47	0.93	1.012	5.82	1.01	1.023
Tulobuterol	5.12	0.51	1.010	5.40	0.60	1.010	5.71	0.65	1.011
Clorprenaline	4.88	1.34	1.018	5.25	1.50	1.020	5.61	1.49	1.023
Terbutaline	5.17	1.05	1.012	5.97	1.31	1.017	6.21	1.40	1.020

BGE: 10.0 mmol L^{-1} HDHSA-β-CD in phosphate buffer, pH 2.5, 20 kV;
The calculations of enantioseparation parameters of CE are the same as in Table S1.

Effect of voltage

To improve the speed and resolution of the developed method, voltage was investigated under the above selected conditions. Table S4 depicts the effect of voltage on the separation obtained at 10, 15 and 20 kV. It can be seen that an increase in the separation voltage produced a decrease in the migration time and also in the resolution. Thus the optimum voltage for the enantioseparation was 10 kV. Under the above selected conditions (pH 2.5,

10.0 mmol L^{-1} HDHSA-β-CD concentration, 60.0 mmol L^{-1} sodium phosphate buffer and 10 kV separation voltage), racemic Clenbuterol, Clorprenaline and Terbutaline were achieve chiral separation completely, and Tulobuterol reached chiral separation partly.

Table S4. The effect of the voltage on the t_1(min), R and α of four analytes.

V	**20**			**15**			**10**		
(kV)	t_1	R	α	t_1	R	α	t_1	R	α
Clenbuterol	5.47	0.93	1.012	10.55	1.48	1.018	18.08	2.20	1.031
Tulobuterol	5.40	0.60	1.010	10.27	1.11	1.016	17.90	1.16	1.017
Clorprenaline	5.25	1.50	1.020	10.04	2.43	1.031	17.26	2.59	1.035
Terbutaline	5.97	1.31	1.017	11.19	2.15	1.028	19.74	2.55	1.036

BGE: 10.0 mmol L^{-1} HDHSA-β-CD in 60.0 mmol L^{-1} phosphate buffer, pH 2.5;
The calculations of enantioseparation parameters of CE are the same as in Table S1.

Clenbuterol

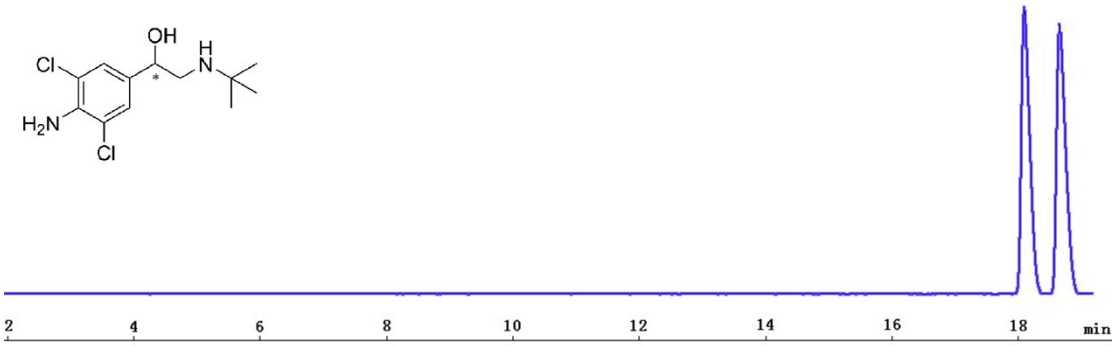

Figure S1. Electropherogram and chemical structure of racemic clenbuterol separated by capillary electrophoresis with HDHSA-β-CD as chiral selector.

Tulobuterol

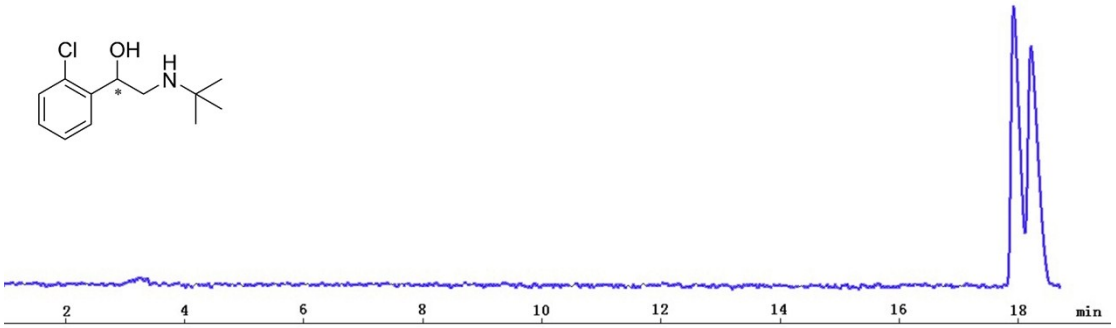

Figure S2. Electropherogram and chemical structure of racemic tulobuterol separated by capillary electrophoresis with HDHSA-β-CD as chiral selector.

Clorprenaline

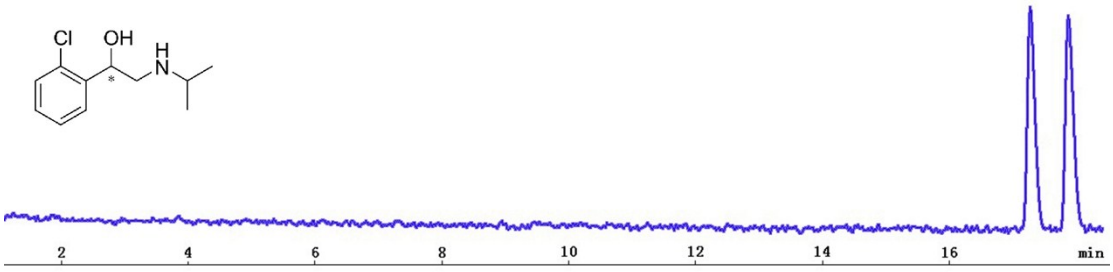

Figure S3. Electropherogram and chemical structure of racemic cloprenaline separated by capillary electrophoresis with HDHSA-β-CD as chiral selector.

Terbutaline

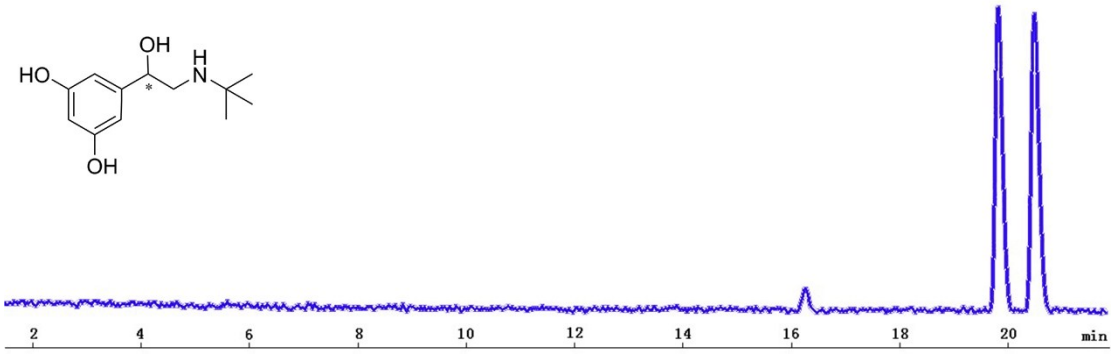

Figure S4. Electropherogram and chemical structure of racemic terbutaline separated by capillary electrophoresis with HDHSA-β-CD as chiral selector.

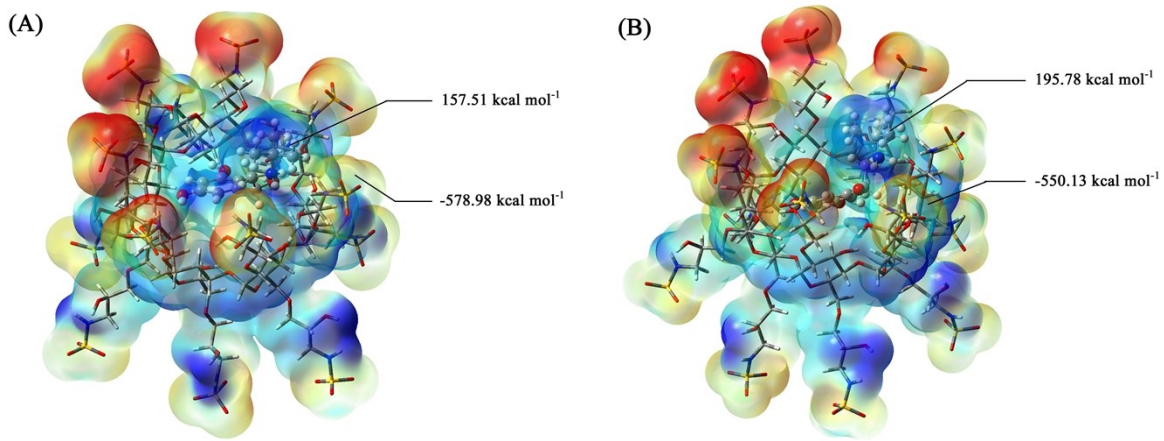

Figure S5. Molecular electrostatic potential maps of *R*-terbutaline/HDHSA-β-CD complex (A) and *S*-terbutaline/HDHSA-β-CD complex (B).

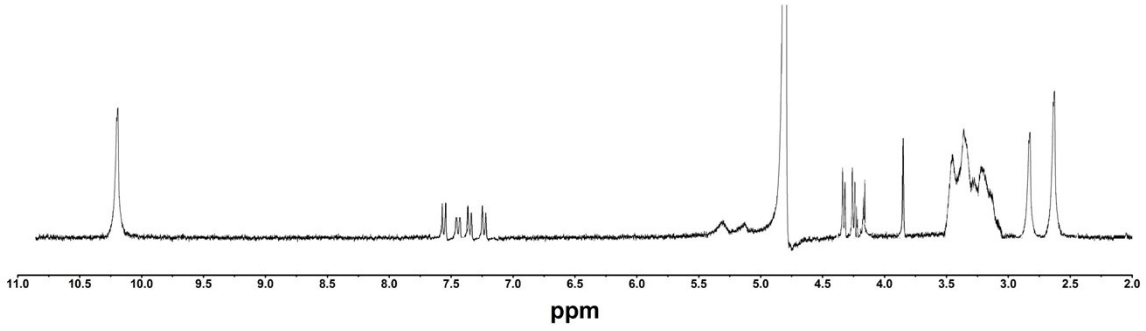

Figure S6. The ^{1}H NMR spectrum of HDHSA-β-CD.