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A strategic approach to the development of capillary electrophoresis chiral methods for pharmaceutical basic compounds using sulfated cyclodextrins

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Dedicated to Professor Dr Gottfried Blaschke on the occasion of his 65th birthday.

Abstract

Enantioseparations of basic pharmaceutical compounds were investigated using different types of sulfated cyclodextrins as chiral selectors. A general strategy for method development was described, together with enantiomeric separations of a number of pharmaceutical related compounds. Based on this strategy, systematic method development approaches for several selected compounds were performed by modifying method parameters, such as the concentration of the chiral selectors, buffer pH, type of organic modifiers, buffer type, temperature and applied voltage. The results of the investigation elucidated the separation mechanism. Many practical aspects were also discussed through several specific examples in order to demonstrate how to develop and validate a precise, sensitive, accurate and rugged separation.

Keywords: Capillary electrophoresis; Sulfated-β-cyclodextrins; Pharmaceutical compounds; Method validation; Enantiomeric separations

1. Introduction

In the past 15 years, capillary electrophoresis (CE) has been established as a tool for enantiomeric analysis, in addition to enantioselective GC and HPLC. The applications of CE chiral separation have been extensively reviewed [1–5].

A CE chiral separation can be achieved by using chiral selectors, such as chiral crown ethers, chiral micelles, proteins and cyclodextrins (CDs) or their derivatives. Cyclodextrins (CDs) are the most widely used chiral selectors. Recently, charged CDs have provided additional alternatives towards the development of fast, simple and efficient CE enantiomeric separation methods. Vigh et al. developed a mathematical model—charged resolving agent migration (CHARM)—for CE enantiomeric separations, using negatively charged single isomeric CDs [6]. Others have utilized Wren and Rowe's model to describe the

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behavior using randomly substituted charged CDs [7].

In this paper, a general chiral CE method development strategy, utilizing charged CDs together with successful enantiomeric separation of a number of pharmaceutically related compounds, is presented. Many practical aspects are discussed through specific examples, in order to demonstrate how to develop and validate a precise, sensitive, accurate and rugged separation.

2. Experimental

2.1. Instrumentation

A Hewlett-Packard ^{3}D capillary electrophoresis (HP ^{3}D, HPCE, Hewlett-Packard Co., Wilmington, DE) system was used. Three different lengths of fused-silica capillaries (56, 41.5 and 26 cm effective length, 63, 48.5 and 33 cm total length with 75 μm internal diameter) were purchased from Hewlett-Packard. Data collection and analysis was performed with a PE Nelson data system equipped with Turbochrom software (PE Nelson, Cupertino, CA).

2.2. Reagents

Compounds A, B, C, D, E, F, G, H, I, J, K, L, M, N, P, Q (Fig. 1) were prepared by the Process Research Department of Merck Research Laboratories (Rahway, NJ). Compound O (Fig. 1) was purchased from Aldrich (Milwaukee, WI). Heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin (HDAS-β-CD), heptakis-(2,3-dimethyl-6-sulfato)-β-cyclodextrin (HDMS-β-CD) and heptakis-6-sulfato-β-cyclodextrin (HS-β-CD) were purchased from Regis (Morton Grove, IL). Octakis (2,3-diacetyl-6-sulfato)-γ-CD (ODAS-)-γ-CD was purchased from J&W Scientific (Folsom, CA). Randomly sulfated-β-cyclodextrins (A-S-β-CD) and α-CD (A-S-α-CD) were purchased from Aldrich Chemical Co. Randomly sulfated β-cyclodextrins (B-S-β-CD) was purchased from Beckman Coulter Inc. (Fullerton, CA). Another type of randomly sulfated-β-cyclodextrins (C-S-β-CD) was obtained from Cerestar USA, Inc. (Hammond). Phosphate buffers (50 mM, pH = 2.5 and 7.0), Tris buffer (50 mM, pH = 8.3), Tris-borate buffer (pH = 9.3), 1 and 0.1 N sodium hydroxide (NaOH) were purchased from Hewlett-Packard. Methanol (MeOH), acetonitrile (ACN) and triethylamine (TEA) were obtained from Fisher Scientific (Springfield, NJ). HPLC grade phosphoric acid was obtained from Aldrich Chemical Co. Deionized water was obtained from a Milli-Q system (Millipore, Bedford, MA).

2.3. Solutions

The diluent for all solution preparations was 90:10 (0.1% H_3PO_4:acetonitrile). All enantiomeric mixture standard solutions were made by mixing appropriate amounts of the pure enantiomers. The recovery test solutions contained the test compounds spiked with 0.1, 0.5 and 1.0 wt% of the minor enantiomer. The neutral marker of electroosmotic flow (EOF) was methanol. All solutions were filtered through a 0.45 μm Nylon-66 membrane syringeless filter device (Whatman, Clifton, NJ).

2.4. Capillary preconditioning

Fused silica capillaries were flushed with 1 N NaOH for 60 min followed by deionized water for 10 min and 0.1 N NaOH for 30 min.

2.5. Electrophoretic separation conditions

Except when systematically varying the composition, the background electrolyte (BGE) used was 25 mM sodium phosphate buffer (pH = 2.5) with different concentrations of Aldrich sulfated β-CD. A diode array UV detector was set at wavelength 200 nm. Hydrodynamic sample injection was used with a 3-s injection time for the sample solution, then 4-s injection time for the running buffer at 50 mbar pressure. The applied voltage was -10.5 kV ('reversed' polarity) and the capillary temperature was controlled at 20 ± 0.1 °C, unless otherwise specified. The capillary was flushed/conditioned with the BGE for 10 min between injections.

3. Results and discussion

3.1. Method development strategy

After evaluating several compounds (Fig. 1), a general CE-chiral method development strategy for basic enantiomeric compounds was designed. Several factors were considered, including method selectivity, sensitivity, precision, accuracy and cost. In the following sections, each parameter was discussed through specific examples.

3.1.1. Selection of initial conditions

The first parameter considered in affecting a separation was the selection of a suitable CD. Currently, two types of sulfated cyclodextrins, randomly sulfated and single isomer CDs, are commercially available. The characteristics and suppliers of those sulfated CDs are listed in Table 1.

Since the single-isomer sulfated CDs were reported to provide more rugged separation selectivity from batch to batch [6], an initial attempt

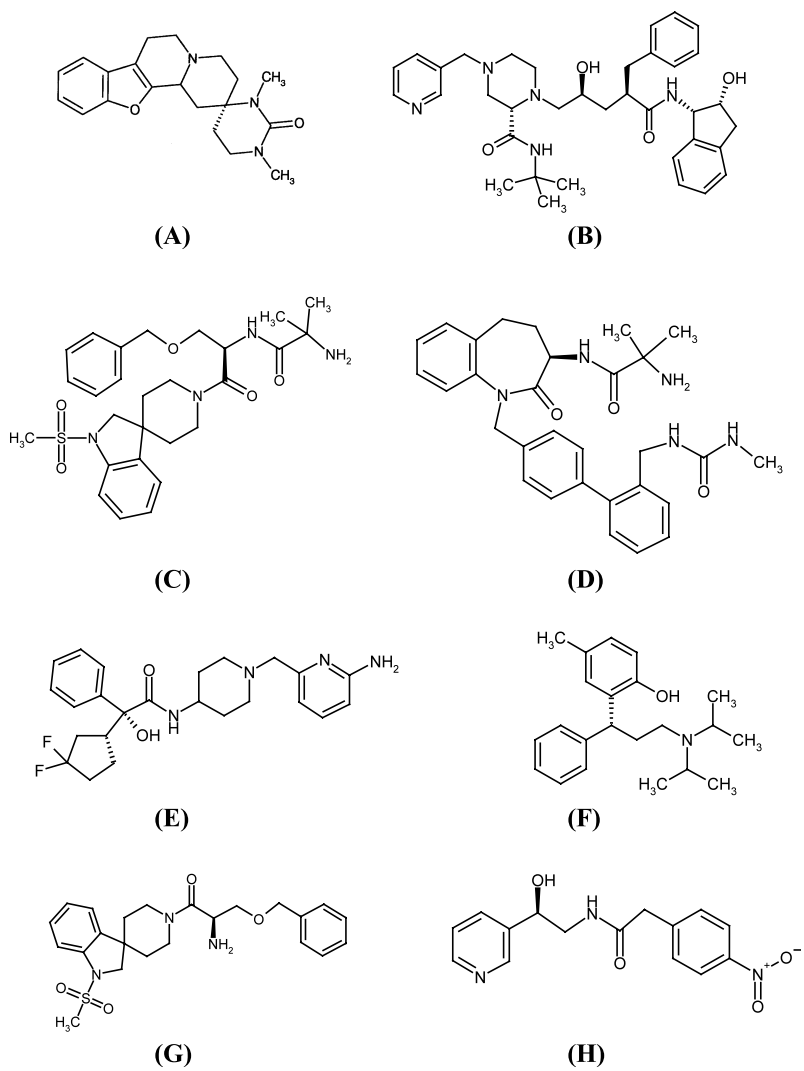

Fig. 1. The structures of different drug-related chiral compounds. Compounds A–F are drug substance candidates. Compounds G–Q are synthetic intermediates or key raw materials for the drug substances.

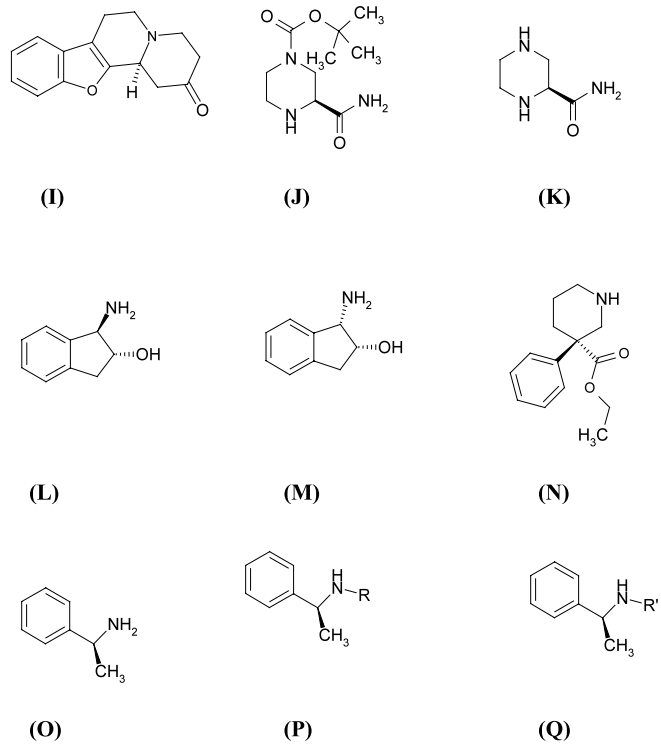

Fig. 1. (*Continued*)

was made using these sulfated CDs. However, the results were not as expected. The chiral separation of many samples suffered from either poor efficiency or selectivity.

Table 2 presents a parallel comparison study for five pairs of selected enantiomers using different sulfated β-CDs. These enantiomeric pairs possessed varied molecular size, amine type, pK_a values and structural rigidity. The results of the study indicated that, under the same operational conditions, the randomly sulfated β-CDs from Aldrich (A-S-β-CD) and from Beckman Coulter (B-S-β-CD) gave identical separation selectivity with similar current and were superior in comparison to all single-isomer sulfated β-CDs and the sulfated β-CD from Cerastar (C-S-β-CD). A comparison electropherogram of compound A with various sulfated CDs is shown in Fig. 2. Compound L was re-injected under normal polarity using all three single isomer CDs, as no peaks were observed under reversed polarity. Peaks were observed in these new runs with long migration times (≈ 40 min), but only heptakis-6-sulfato-β-cyclodextrin (HS-β-CD) gave separation. These observations indicated that binding between the selected compounds and single isomer CDs were weaker than with the randomly sulfated CDs. The fact that the [compound L:single isomer CDs] complexes were still cationic provided good evidence. The stronger interaction could be attributed to the position of substitution on CDs. The randomly sulfated CDs were substituted at both C2 and C6 position [8] and this could be responsible for the additional interactions.

The purity of the CDs was also determined. An indirect-UV CE method [9,10] was used to determine the distributions of the degree of sulfation and overall purity of A-S-β-CD and B-S-β-CD. Heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin (HDAS-β-CD) was used as a control sample. The results revealed that A-S-β-CD, B-S-β-CD and HDAS-β-CD each gave a major single peak with

Table 1
Commercially available sulfated CDs

CD-type	Supplies	Average sulfates/molecules
Random sulfated β-CD(A-S-β-CD)	Aldrich	7–11
Random sulfated β-CD(C-S-β-CD)	Cerastar	4
Random sulfated β-CD(B-S-β-CD)	Beckman Coulter	12
Single isomer sulfated β-CD(HS-β-CD)	Regis	5
Single isomer sulfated β-CD(HDAS-β-CD)	Regis	5
Single isomer sulfated β-CD(HDMS-β-CD)	Regis	5
Random sulfated α-CD(A-S-α-CD)	Aldrich	NA
Random sulfated γ-CD(B-S-γ-CD)	Beckman Coulter	13
Single isomer sulfated γ-CD(ODAS-γ-CD)	J&W	8

HS, heptakis-6-sulfato; HDAS, heptakis-(2,3-diacetyl-6-sulfato); HDMS, heptakis-(2,3-dimethyl-6-sulfato); ODAS, octakis(2,3-diacetyl-6-sulfato); NA, not available.

purity >95 area%, revealing homogeneous sulfation for these randomly sulfated CDs. However, the C-S-β-CD contained multiple peaks, which indicated multiple degrees of sulfation, giving rise to different types of possible interactions. This multiplicity may account for its poorer resolving capabilities relative to the other randomly sulfated CDs. For the selected compounds, randomly sulfated A-S-β-CD was better than the other sulfated CDs in terms of selectivity and CD quality. Therefore, A-S-β-CD was selected as the starting chiral selector for routine method development.

3.1.2. Selectivity as a function of CD type

Most of the enantiomeric compounds were resolved using the A-S-β-CD chiral selector (Table 3). However, if A-S-β-CD did not provide satisfactory separation, other sulfated CDs could be considered. For example, in the case of compound E, the B-S-γ-CD showed better selectivity. In the case of compound B, A-S-α-CD and HDAS-β-CD showed better selectivity.

3.2. Method optimization

Once the initial separation was obtained, optimization was achieved by modifying selected parameters, such as the buffer pH, concentration of the chiral selectors, type and amount of organic modifiers, type and concentrations of buffer, capillary size, temperature and applied voltage.

3.2.1. Effect of sulfated CD concentration on the separation

The effect of the concentration of sulfated CDs on the separation was the recommended first parameter for optimization. The binding constants between the charged chiral selector and the enantiomers could not be determined based on

Table 2
Enantiomeric resolution (Rs) for the five selected chiral compounds using different types of sulfated β-CDs

Compound/β-CD type	A-S-	B-S-	C-S-	HS-	HDAS-	HDMS-
A	5.3	5.3	1.8	0.5	0.4	0.1
C	2.4	2.4	1.7	1.1 with splitter peaks	No peak in 30 min	No peak in 30 min
H	2.0	2.0	–	0	No peak in 30 min	No peak in 30 min
L	7.6	7.6	No peak in 30 min	No peak in 30 min	No peak in 30 min	No peak in 30 min
Q	2.0	2.0	2.0	0.1	0	0.1

Rs = 0 was confirmed by spiking both enantiomers. Conditions: Capillary: fused-silica 48.5 cm (effective length 41.5 cm) × 75 μm ID at 20 °C. BGE: 25 mM sodium phosphate buffer, pH = 2.5. Injection: 50 mbar pressure, 3 s for the samples, 4 s for the running buffer. The concentration of each CDs fixed at 2.5%. Applied voltage: −10.5 kV. UV wavelength was 200 nm, except 220 nm was used for the HDAS-β-CD.

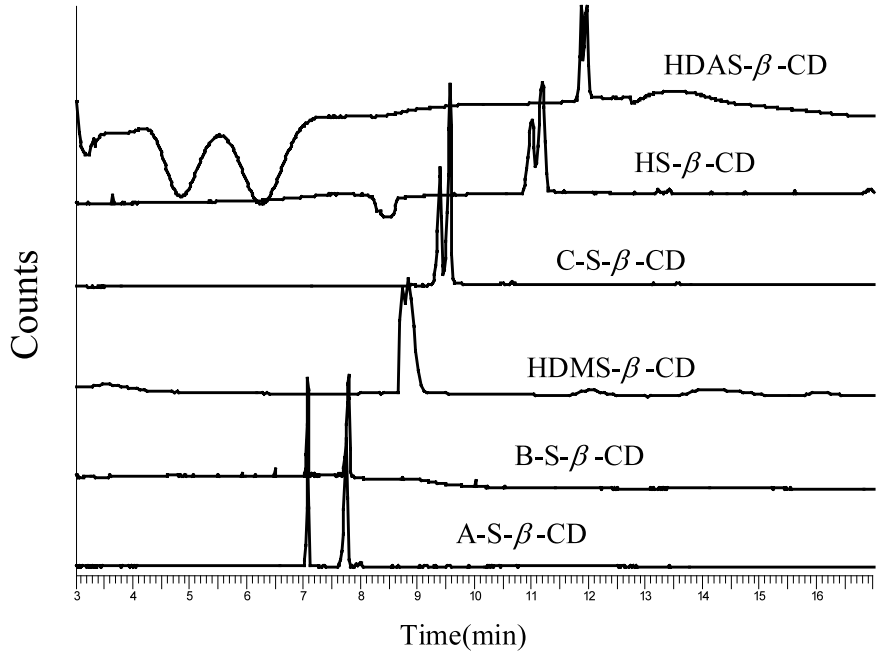

Fig. 2. A comparison electropherogram of compound A with various sulfated CDs. Conditions: Capillary: fused-silica 48.5 cm (effective length 41.5 cm) × 75 μm ID at 20 °C. BGE: 25 mM sodium phosphate buffer, pH = 2.5. Injection: 50 mbar pressure, 3 s for the samples, 4 s for the running buffer. The concentration of each CDs fixed at 2.5%. Applied voltage: −10.5 kV. UV wavelength was 200 nm, except 220 nm was used for the HDAS-β-CD.

the CE data due to the increase of the ionic strength at increasing CD concentrations. However, the optimized CD concentrations could be determined based on the separation selectivity ~ [CD] curves.

The separation selectivity (α) is defined as $\alpha = \mu_1^{\text{eff}}/\mu_2^{\text{eff}}$ (where μ_1^{eff} and μ_2^{eff} are the effective mobility of the two enantiomers) [6,10–13]. As shown in Fig. 3, separation selectivities changed from 1 to a negative value, crossed over to a positive value and then approached its limiting value. The peak separation selectivity reached a maximum close to the net cationic-to-anionic cross-over point. This point was where the net effective charge of free plus complexed analyte changed from positive to negative. The run time was excessive and ruggedness suffered at this maximum because of inefficient peak shape and large variations in migration time and selectivity due to small CD concentration changes. Therefore, concentrations in the middle of the plateau region of the selectivity ~ [CD] curve were selected for the optimized separations. Importantly, with this selection, concentrations of ± 0.5 wt% units provided almost equivalent separations indicating ruggedness.

3.2.2. Effect of the BGE pH on the separation

The selection of the buffer pH should be performed based on the separation type. The pK_a of the compounds listed in Fig. 1 ranged from 5 to 8.5. The enantiomeric separations of those compounds were characteristic of either type II or type III in the CHARM model [6], since they were all resolved at the pH 2.5 BGE, where all compounds were present in fully cationic form. The effective mobility of the enantiomer transitions from cationic to anionic as it complexes with the A-S-β-CD and peaks were eluted on the anode end (reversed polarity setting).

The apparent elution orders of type II and III basic compounds can easily be reversed by simply reversing the applied polarity or changing the pH of the BGE. For example, as shown in Fig. 4, in

the pH 2.5 buffer, the compound L is cationic (pK_a of compound L = 8.5), the effective mobility of both enantiomers becomes anionic upon complexation with A-S-β-CD at 3% (wt%). Therefore, the more strongly bound enantiomer eluted first at the reversed polarity setting. Between pH 7 and 8.3, the net positive charge of compound L was reduced, but the effective mobility of enantiomer A-S-β-CD complexes were still anionic. With a normal polarity, both anionic peaks were dragged by the strong EOF. The stronger-binding complex peak carried a higher negative charge, hence it eluted later and the elution order was reversed [14].

Table 3
The chiral resolution for the basic compounds

Compound	Resolution	CE conditions
A	5.3	a
B	2.0/3.2	b/c
C	2.4	a
D	4.5	a
E	1.2/2.0	a/d
F	2.0/1.8	a/c
G	2.5	a
H	2.0	a
I	5.0	a
J	2.1	a
K	1.8	a
L	7.6	a
M	2.5	a
N	2.5	a
O	1.8	a
P	2.0	a
Q	2.0	a

Conditions: 48.5 cm fused silica capillary, UV detection at $\lambda = 200$ nm and -10.5 kV were used for all separations, except the BGEs, which were varied as follows: a: 2.5% A-S-β-CD in 25 mM, pH = 2.5 phosphate buffer; b: 2.5% A-S-α-CD in 25 mM, pH = 2.5 phosphate buffer; c: 2.5% HDAS-β-CD in 25 mM, pH = 2.5 phosphate buffer; and d: 2.0% B-S-γ-CD in 25 mM, pH = 2.5 phosphate buffer.

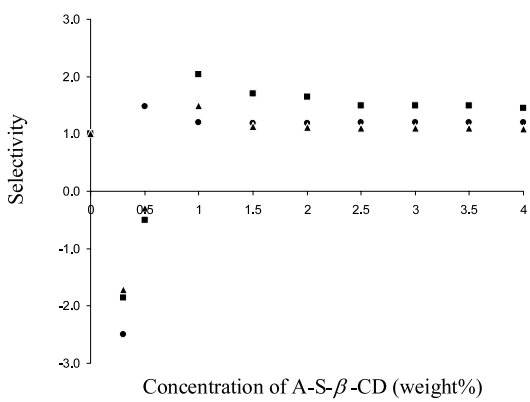

Fig. 3. Effect of the concentration of A-S-β-CD on the enantiomeric separation selectivities of compounds A, C and L. Solid circles (●) are for compound A, solid squares (■) are for compound L and solid up-triangles (▲) are for compound C. Conditions are the same as in Fig. 2, except the concentration of the A-S-β-CD was varied from 0 to 4.0% (wt%).

In the enantiomeric analysis by LC, it is typically desired to have the minor enantiomer eluting before the major enantiomer, in order to minimize quantitation errors due to peak overlap. However, in CE-chiral separation, due to the inherent high efficiency, this order is not a requisite, since in most cases, the peaks are well resolved. Therefore, using low pH BGE is preferred rather than considering the elution order, since the former provide reproducible migration time due to suppression of the EOF.

3.2.3. Effect of the organic modifiers on the separation

The addition of organic modifiers to the BGE can alter several parameters, such as the EOF, the viscosity, the conductivity of the BGE and the binding constant of the CD:enantiomer complexes. It was recognized that organic modifiers compete with analyte for the relatively hydrophobic cavity of CDs. Therefore, the binding between the analyte and the CD was reduced. Several research groups reported that the addition of organic modifiers decreased the CD–analyte binding constants [7,13]. Gratz et al. measured the binding constant between terbutaline and A-S-β-CD by UV and CE. They concluded that the enantioselectivity increased with the addition of acetonitrile and methanol due to a decrease of the binding constant.

The results shown in Fig. 5 are in good agreement with the results observed by others [7]. The trends of the selectivity ~ [CD] curves for compound A with two BGEs were similar except that the selectivity value was slightly higher and reached its plateau at higher CD concentration

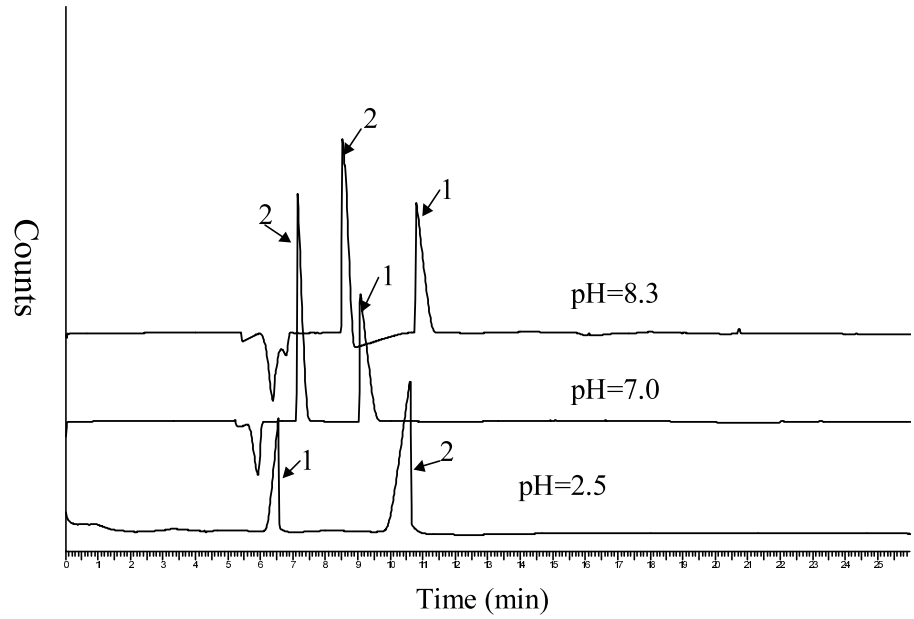

Fig. 4. Effect of the pH on the separation and the elution order for compound L. Conditions: Capillary: fused-silica 33 cm (effective length 26 cm) × 75 μm ID at 20 °C. The BGEs used for the upper and middle electropherograms were 3% (wt%) A-S-β-CD in pH 8.3 Tris borate buffer (25 mM) and in pH 7.0 sodium phosphate buffer (25 mM), respectively, The applied voltage was + 8, the BGE used for the bottom electropherogram was 3% (wt%) A-S-β-CD in pH 2.5 sodium phosphate buffer (25 mM) and the applied voltage was − 8 kV. Peak 1 is the minor enantiomer of the compound L and peak 2 is the compound L.

when the 10% ACN was added to the BGE. The observation may be attributed to weakening of the affinity of the analytes for A-S-β-CD with the addition of the acetonitrile, leading to a shift of the selectivity curve towards higher A-S-β-CD concentrations. Consequently, for a given CD concentration, the selectivity increased although the peaks were broader.

One important practical reason to add the organic modifier into the BGE was to enhance the solubility of the studied compounds. For example, compound Q has poor solubility in water due to its large size hydrophobic side chain. The addition of the acetonitrile into the BGE allowed for greater solubility.

3.2.4. Effect of buffer concentration and buffer type on the separation and the detection signal-to-noise ratio

Since all sulfated CDs are fully ionized in the aqueous buffer solution, they contribute significantly to the ionic strength of the BGE. There-

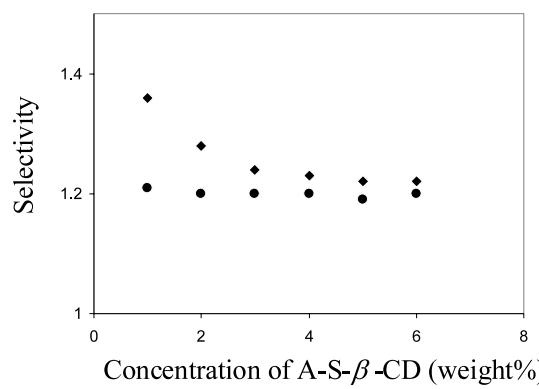

Fig. 5. Effect of acetonitrile on the selectivity of compound A and its enantiomer. Conditions: BGE for solid circle (●) curve: pH 2.5 sodium phosphate buffer (25 mM), BGE for the solid diamond (◆) curve: pH 2.5 sodium phosphate buffer (25 mM) with 10% acetonitrile. A-S-β-CD concentration: varied from 0 to 6.0% (wt%). Capillary: fused-silica 33 cm (effective length 26 cm) × 75 μm ID, temperature: 20 °C. Applied voltage: − 8 kV.

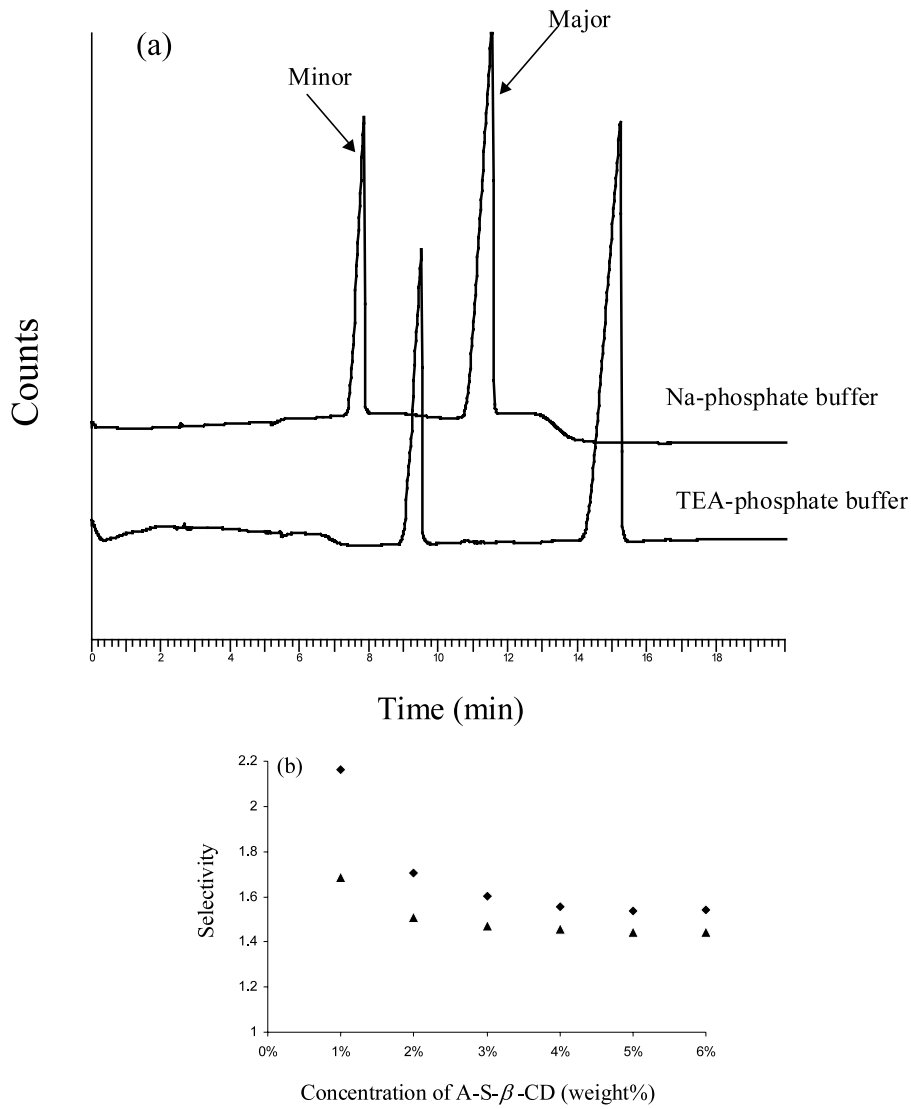

Fig. 6. (a) Electropherograms of compound L and its enantiomer using different buffers. Conditions: BGEs for upper electropherogram: pH 2.5 sodium phosphate buffer (25 mM), BGE for bottom electropherogram: pH 2.5 TEA-phosphate buffer (25 mM), concentration of the A-S-β-CD: 3%. (b) Effect of the buffer type on the separation selectivity. BGE for solid upper triangle (▲) curve: pH 2.5 sodium phosphate buffer (25 mM), BGE for solid diamond (◆) curve was pH 2.5 TEA-phosphate buffer (25 mM). Concentration of A-S-β-CD: varied from 0 to 6.0% (wt%). Other conditions of both figures are the same as Fig. 5.

fore, the change in concentration of the sodium phosphate buffer from 15 to 50 mM had an insignificant impact on the effective mobility and resolution. However, changing the buffer counter ion from sodium to triethylamine (TEA) at the same molar concentration and pH had a significant influence on the separation of compound L. As shown in Fig. 6(a), the resolution increased while the effective mobility of the compound L and its enantiomers decreased when utilizing the TEA-phosphate BGE. Vincent et al. measured the EOF for the TEA-phosphate BGE with and without HDAS-β-CD [15]. Their results revealed significant TEA adsorption on the capillary wall

with the absence of the HDAS-β-CD. The EOF was anionic initially, but as the concentration of HDAS-β-CD increased, the EOF became cationic. Since the concentration of A-S-β-CD was elevated in both BGEs in this study, the TEA effect due to absorbance on the wall should be negligible. In addition, under 'reversed' polarity, the effective mobilities of the anionic complexes would increase if an anionic EOF was present.

To further investigate the effect, an additional experiment was performed using both TEA-phosphate and Na-phosphate buffers for compound L. The concentration of the A-S-β-CD was varied from 1 to 6%. The results are shown in Fig. 6(b). The trends of the selectivity versus A-S-β-CD concentration were similar for both buffers, but the selectivity obtained by TEA-phosphate buffer was consistently larger than in the Na-phosphate buffer over the studied concentration range. This behavior was similar to the effect with acetonitrile on the selectivity shown in Fig. 5. Therefore, the separation behavior may be attributed, in part, to TEA competing with the analyte for the relatively hydrophobic cavity of A-S-β-CD, as well as through ion-pairing [16] resulting in reduced complexation. As a consequence, the overall negative charge was reduced, the anionic effective mobility decreased and the selectivity increased due to the shifting of its maximum value towards higher CD concentrations.

Table 4
Effect of BGE type on the S/N ratio of compound L at the 0.1% level

BGE type	S/N ratio at 0.1% level	
	($\lambda = 200$ nm)	($\lambda = 220$ nm)
Sodium phosphate (pH = 2.5)	45	16
Triethyl amine-phosphate (pH = 2.5)	19	8
Triethyl amine-acetate (pH = 3.0)	5	2
Triethyl amine-citrate (pH = 3.5)	4	3

Conditions: 48.5 cm fused silica capillary, UV detection at $\lambda = 200$ nm and -15 kV were used for all separations except the BGEs, which were varied as indicated in the table. The target concentration of the compound L was 0.3 mg/ml.

One of the major concerns for selection of a buffer system is the UV cut off. Small detection path lengths give rise to detection sensitivity issues and thus, BGE with low background UV absorbance is preferred to enhance sensitivity. Several buffer systems have been used for BGE preparation. Signal to noise levels of compound L were compared using BGE containing Na-phosphate, TEA-phosphate, TEA-acetate and TEA-citrate, as shown in Table 4. The results clearly demonstrated that the Na-phosphate buffer was the best choice in terms of UV detection sensitivity.

3.2.5. Effect of the temperature and other operational parameters on the separation

The capillary temperature can affect the mobility of the analytes and the thermodynamics of the binding of the enantiomers with CDs. A plot of $\ln \alpha$ versus $1/T$ was linear with $r^2 = 0.99$ for compounds A, C and L in the temperature range from 5 to 40 °C. The effect of the temperature was consistent with the literature [12,17]. Kuhn and Erni estimated the thermodynamic values of the enantiomeric separation on CE using crown ether as chiral selector based upon the linear plot of $\ln(\alpha)$ versus $1/T$ [12]. However, it is still controversial as to whether the direct application of the '$\log \alpha$ versus $1/T$' as a Van't Hoff plot is appropriate, as the CE process is dependent upon the current, especially when charged chiral additives are used. It can be speculated that the linearities reflect the constancy over the temperature range of the interactions of the enantiomers with the A-S-β-CD [17]. Additionally, resolution increased as the temperature decreased with a parallel sacrifice in run time and poorer peak shape. A compromise temperature should be set case by case.

The capillary length can affect the analysis time significantly. The run time can be reduced using a shorter capillary with little change in the resolution when a constant electric field is applied.

Effective mobilities of the analytes should be independent of the applied voltages. The effective mobilities were almost constant in the range from 5 to 20 kV for most separations. However, they

started to increase when the voltage was set higher than 20 kV. This behavior was due to the excessive Joule heating generated during the electrophoresis process because the negatively charged sulfated CDs had a large contribution to the current.

3.3. Method validation

Appropriate validation of an analytical method is very important in the pharmaceutical industry. The purpose of validation is to fully demonstrate that the method is suitable for its intended use. It usually covers several important analytical criteria, such as sensitivity, selectivity, detection linearity, precision, accuracy and ruggedness.

3.3.1. Sensitivity

As discussed in the previous sections, the low method sensitivity is a potential disadvantage of the CE technique. However, this is also a challenge for LC chiral separation, where peak efficiencies for the commonly used columns are low. A suitable sensitivity method is achieved by setting the UV wavelength as low as possible, whether by LC or CZE. Thus, the UV cut off of the mobile phase in LC and BGE in CZE should be as low as possible. In addition, for the CZE technique, the capillary internal diameter should be as large as possible to maximize path-length.

The method sensitivity can be demonstrated by the LOD. At the concentration of LOD, the injected solution should produce a signal to noise ratio of 3 [18].

The limit of quantitation can be determined based on satisfying three criteria: (a) an S/N ratio of the LOQ solution >10; (b) a percent difference of response factor values at the LOQ and $5\times$ LOQ <20; and (c) a R.S.D. of area counts for three injections at LOQ level $<15\%$ [10,18].

Fig. 7(a,b) demonstrates examples of LOQ determinations. As can be seen from the figures, the minor enantiomer peak of compound L at 0.05% level can be clearly detected on the front or back of the major peak with a signal to noise ratio >20.

3.3.2. Lot to lot reliability of the random sulfated CDs

One of the major concerns in using the random sulfated CDs is lot to lot reliability. The migration time and selectivity changes of compound C were evaluated using different lots of A-S-β-CD. The results shown in Table 5 demonstrate that the changes in both parameters are negligible.

3.3.3. Precision and accuracy

Typical injection precision data were presented in Table 6. Pure compound C was spiked with its enantiomer at 0.5% (wt%) level, then the solution was injected six times consecutively. The % R.S.D. of quantitation was 0.02% for compound C and 3.9% for the minor enantiomer based on the area percent.

Accuracy is usually determined by calculating the recovery of the spiked minor enantiomer at different levels or by comparison with an independent LC method. The average recoveries for the compound C was 103%.

3.3.4. Ruggedness

The ruggedness of the method reflects the ability of the method to be performed by different chemists on different instruments with different capillary lots on different days. Very reproducible results in terms of the migration time, separation selectivity and resolutions were achieved for the chiral separation of compound C, L and Q with all of these variations. The performance was sometimes superior to LC chiral column separation methods. The migration times of analytes gradually increased and the separation efficiency decreased as the same vial of the running buffer was repeatedly used. Therefore, it is recommended to change the running buffer after every two runs.

4. Conclusions

A simple, fast, accurate, precise, rugged and sensitive chiral CE method with desired selectivity can be developed using sulfated CDs through a systematic approach to method development based on a general strategy. The enantiomeric

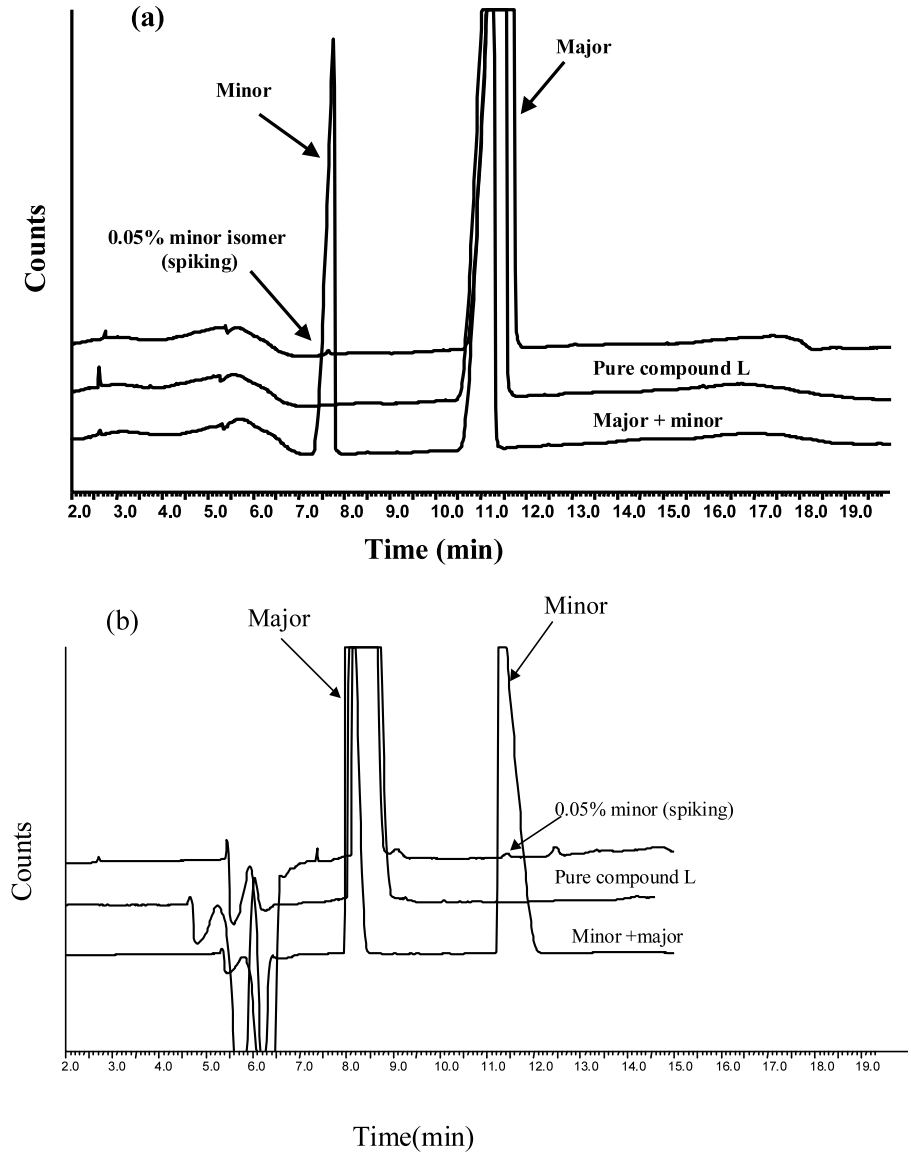

Fig. 7. (a) Electropherogram of pure compound L and pure compound L spiked with 0.05% of the minor enantiomer in pH 2.5 buffer. Conditions: pH 2.5 sodium phosphate buffer (25 mM). Capillary: fused-silica 33 cm (effective length 26 cm) × 75 μm ID, temperature: 20 °C. Applied voltage: −8 kV. UV detection, $\lambda = 200$ nm. (b). Electropherogram of pure compound L and pure compound L spiked with 0.05% of the minor enantiomer in pH 7.0 buffer. Conditions are the same as (a) except the pH of the buffer was 7.0 and the applied voltage was +8 kV.

purity of drug-related compounds can be quantitatively determined. Future work will investigate a structural relationship between the chiral compounds and the different CDs by CE, in order to elucidate detailed separation mechanisms.

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Table 5
Lot to lot variability of the A-S-β-CD

Lot No.	Injection No.	MT-1C	MT-2C
JI05706 HI	HI-1	12.456	14.467
	HI-2	12.401	14.023
HU05208HU	HU-1	12.356	14.235
	HU-2	12.405	14.256
Average		12.404	14.244
% R.S.D.		0.3	1.3

Conditions: pH 2.5 sodium phosphate buffer (25 mM), Capillary: fused-silica 33 cm (effective length 26 cm) × 75 μm ID, temperature: 20 °C, applied voltage: −8 kV. UV detection at $\lambda = 200$ nm. Key: MT-1C: migration time of the first eluted peak of compound C, MT-2C: migration time of the second eluted peak of compound C.

Table 6
The injection precision of the chiral method for the compound C

Replicate injections number	Area % of major isomer	Area % of minor isomer
1	99.46	0.54
2	99.46	0.54
3	99.42	0.58
4	99.43	0.57
5	99.47	0.53
6	99.47	0.53
Average	99.45	0.55
% R.S.D.	0.02	3.90

Conditions are the same as Table 5.

References

[1] B. Chankvetadze, C. Blaschke, J. Chromatogr. A 906 (2001) 309–363.
[2] C. Blaschke, B. Chankvetadze, J. Chromatogr. A 875 (2000) 3–25.
[3] S. Fanali, J. Chromatogr. A 875 (2000) 89–122.
[4] K. Otsuka, S. Terabe, J. Chromatogr. A 875 (2000) 163–178.
[5] B. Chankvetadze, J. Chromatogr. A 792 (1997) 269–295.
[6] B. Williams, G. Vigh, J. Chromatogr. A 777 (1997) 295–309.
[7] S.R. Gratz, A.M. Stalcup, Anal. Chem. 70 (1998) 5166–5171.
[8] J. Chapman, F. Chen, LC-GC Eur. 14 (2001) 33–38.
[9] H. Cai, T. Nguyen, G. Vigh, Anal. Chem. 70 (1998) 580–589.
[10] L. Zhou, D.B. Johnson, C. Miller, J.M. Wyvratt, J. Chromatogr. A 875 (2000) 389–401.
[11] W. Friedland, E. Kenndler, Anal. Chem. 65 (1993) 2003–2014.
[12] R. Kuhn, F. Erni, Anal. Chem. 64 (1992) 2815–2820.
[13] S.G. Penn, D.M. Goodall, J.S. Loran, J. Chromatogr. A 636 (1993) 149–152.
[14] B. Chankvetadze, G. Schulte, G. Blaschke, J. Chromatogr. A 732 (1996) 183–187.
[15] J.B. Vincent, G. Vigh, J. Chromatogr. A 817 (1998) 105–111.
[16] C. Quang, M.G. Khaledi, Anal. Chem. 65 (1993) 3354–3358.
[17] L. Zhou, J. Trubig, A. Dovletoglou, D. Locke, J. Chromatogr. A 773 (1997) 311–320.
[18] US Pharmcopoeia, No. 1225, Validation of Compendial Method, USP Convention Inc., Rockville, MD, 1995, pp. 1982–1984.