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Review

Analytical method for the quantitation of sertraline hydrochloride stereoisomers by electrokinetic chromatography

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Abstract

Sertraline is a basic compound and of pharmaceutical application for antidepressant treatment. The compound has two chiral centers. Separation of the three enantiomeric impurities from the parent compound is challenging. In this study, we successfully separated all four stereoisomers by electrokinetic chromatography using highly sulfated γ-cyclodextrin and highly sulfated α-cyclodextrin as the chiral selectors. The two chiral selectors provided different selectivity and therefore affected the overall separation profiles. This may be due to the size difference between the dichlorophenyl moiety end and naphthalenamine moiety end, resulting in two different types of inclusion complexes with the different cyclodextrins. For routine analysis, highly sulfated γ-cyclodextrin was better than highly sulfated α-cyclodextrin. For each stereoisomeric impurity, the method using sulfated γ-cyclodextrin provided a limit of quantitation at or lower than 0.1% of the drug substance with adequate resolution. The critical resolution at this concentration level was not less than 4.0. Experimental data suggested that an internal standard was necessary for the purpose of quantitation, and the practical linearity range for analysis of sertraline stereoisomeric impurities was of about two orders of magnitude.

Keywords: Sertraline hydrochloride; Chiral separation; Enantiomer separation; Highly sulfated cyclodextrin; Electrokinetic chromatography

Contents

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1. Introduction

Understanding the stereochemistry of pharmaceutical compounds is very important in regard to their biological activities within human body. In 1992, Food and Drug Administration (FDA) has issued a policy statement for the development of new stereoisomeric drugs that requires the acceptable manufacturing control of synthesis and impurities, adequate pharmacological and toxicological assessment, proper characterization of metabolism and distribution, and appropriate clinical evaluation [1]. Adequate separation and accurate quantitation of the enantiomers is consequently required by the regulation agency. This is a challenge to both chemical manufacturing and pharmaceutical industries.

Chromatographic separation such as gas chromatographic (GC) and especially high-performance liquid chromatography (HPLC) has been widely used by the pharmaceutical and chemical industry. The first chromatographic separation of enantoimers was published in 1965 [2]. Since then, thousands of articles have been published on the chromatographic separation of enantiomers. One effective approach to the separation of enantiomers is using a chiral selective stationary phase. However, these chiral stationary phases are usually made by coating the chiral selective material onto the silica bed. These coated stationary phases are not durable and quite expensive compared to a conventional reversed-phase column. The first application of CE to chiral separation was published in 1985 [3]. Since 1992, after the issue of FDA policy on the development of new stereoisomer drugs [1], more and more research papers were published on chiral separation by CE. Since 1995, more than 100 articles per year on chiral separations by CE have been published. In addition to that the operating cost of capillary electrophoresis (CE) is much less than that of HPLC or GC, a CE method is often more efficient and specific. Sometimes, CE methods can be orthogonal to HPLC methods, and other times, CE methods can separate enantiomers that HPLC methods cannot do. Therefore, the use of capillary electrophoresis as the chiral separation technique is of tremendous advantageous.

Among the variety of chiral selectors, cyclodextrin has been recognized as a very effective chiral selector. Cyclodextrin forms an inclusion complex with the analyte and due to the difference of structures and positions, stereoisomers can be separated. Cyclodextrins are neutral, natural cyclic oligosaccharides obtained by enzymatic cleavage of starch. When starch is treated with glycosyltransferases it undergoes hydrolysis furnishing a mixture of oligosaccharides consisting of six up to 13 glucose units [4]. The α-, β-, and γ-CD contain six, seven, and eight glucose units, respectively. Their physical properties are listed in Table 1 [4,5]. The unmodified CDs alone cannot separate the neutral analytes in CE, and the separation window and selectivity of un-modified CDs for stereoisomeric separation of charged analytes are also limited. Therefore, researchers developed charged CDs, especially highly sulfated cyclodextrins that provide substantial selectivity to a wide variety of pharmaceutical compounds [6–28]. Although the exact inner and outer diameters of the cavities of the sulfated CDs are not certain, we can predict that due to the replacement of hydroxy groups (–OH) with sulfate groups ($-SO_4^-$), d_1 is increased and d_2 is decreased, the latter reflecting that the cavity of sulfated CDs is smaller than that of the un-modified CDs.

Table 1
Properties of un-modified cyclodextrin

	α-CD	β-CD	γ-CD
Unit of glucopyranose	6	7	8
Molecular weight (g/mol)[a]	972	1135	1297
Volume of cavity (Å^3)[a]	176	346	510
Solubility in water[a] (g/100 mL at 25 °C)	14.5	1.85	23.2
d_1 (Å)[b]	13.7	15.3	16.9
d_2 (Å)[b] or diameter of cavity	5.7	7.8	9.5
Depth (Å)[b]	7.9	7.9	7.9

[a] Data from [4].
[b] Data from [5].

Sertraline HCl, or *cis*-(1*S*,4*S*)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-nanphthalenamine hydrochloride, is an active pharmaceutical ingredient. Sertraline (Zoloft) is used in the treatment of all types of depression. It may also be used for panic disorder, social phobia, obesity, or obsessive-compulsive disorder (OCD). The empirical formula of sertraline HCl is $C_{17}H_{17}NCl_2{\cdot}HCl$. The molecule has two stereogenic centers. The structures of sertraline hydrochloride and its stereoisomers are shown in Fig. 1. Sertraline is a secondary amine with pK_a of 9.47 ± 0.20 calculated using Advanced Chemistry Development (ACD) Software Solaris V4.67 (1994–2003 ACD©). Separation of sertraline stereoisomers by CE was reported once before [29]. Although the paper claimed the limit of quantitation of sertraline stereoisomeric impurities at a level of 0.1% when the active (*cis*-(1*S*,4*S*)) (**1**) was injected together with its stereoisomeric impurities at a limit of quantitation level, the paper did not demonstrate the separation of sertraline stereoisomeric impurities at 0.1% in the presence of sertraline hydrochloride bulk drug substance. Based upon the resolution between the stereoisomers demonstrated in the paper [29], sertraline enantiomeric impurities could not be possibly separated from sertraline at 0.1% level when the bulk drug substance is analyzed because of the greater resolution needed for the separation of stereoisomers that vary widely in concentration.

In this paper, we focused on developing an analytical method to separate sertraline hydrochloride (**1**) from its stereoisomers by CE technology using electrokinetic chromatography (EKC) with highly sulfated cyclodextrins (HSCDs) as the chiral selectors. The HSCDs were the additive to the background electrolyte, the latter of which served as a pseudo-stationary phase in capillary electrophoresis. We demonstrated that the new developed analytical method provided a much superior selectivity and separation for all sertraline stereoisomers. Quantitation of enantiomeric impurities in the bulk drug substance at 0.1% or even lower can be readily achieved.

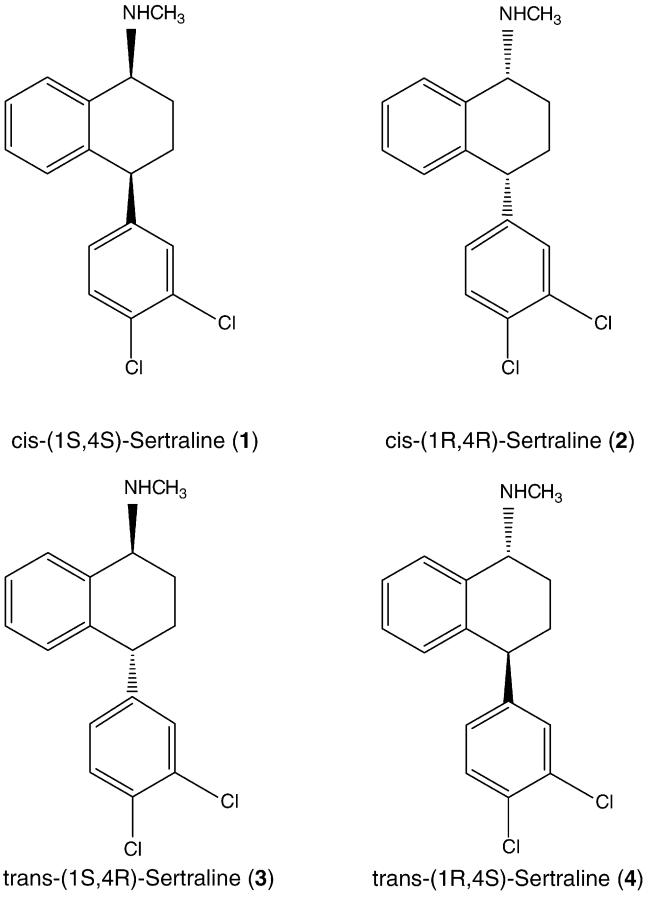

Fig. 1. Chemical structures of sertraline stereoisomers.

2. Materials and methods

2.1. Reagent

Sertraline hydrochloride (*cis*-(1*S*,4*S*)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-nanphthalenamine hydrochloride) or *cis*-(1*S*,4*S*) stereoisomer (**1**), *cis*-(1*R*,4*R*)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-nanphthalenamine hydrochloride or *cis*-(1*R*,4*R*) stereoisomer (**2**), *trans*-(1*S*,4*R*)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-nanphthalenamine hydrochloride or *trans*-(1*S*,4*R*) stereoisomer (**3**), and *trans*-(1*R*,4*S*)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-nanphthalenamine hydrochloride or *trans*-(1*R*,4*S*) stereoisomer (**4**) were purchased from Interchem Corporation, Paramus, NJ, USA. Purified DI water was from a Barnstead E-pure filtration system with a resistance of at least 18 MΩ. Methanol was purchased from Fisher Scientific (Bellefonte, PA, USA). The background electrolyte, 50 mM triethylammonium phosphate buffer at pH 2.5 were purchased from Beckman Coulter (Fullerton, CA, USA). The chiral selectors, highly sulfated α-cyclodextrin (HS-α-CD), highly sulfated β-cyclodextrin (HS-β-CD), and highly sulfated γ-cyclodextrin (HS-γ-CD) with sodium as the counter ion were purchased as 20% (w/v%) aqueous solutions from Beckman Coulter (Fullerton, CA, USA). The average degree of sulfation was 11, 12, and 13 for α-, β-, and γ-HSCD, respectively [19]. The degree of sulfation was determined from the sulfur/carbon ratio. The chiral selector solutions and the 50 mM triethylammonium phosphate buffer solution were kept at about 4 °C for storage.

2.2. Preparation of buffers and samples

All buffers were made in 25 mM of triethylammonium phosphate aqueous solution at pH 2.5 containing 5% (w/v%) of HS-CE aqueous solution by mixing 50 mM of triethylammonium phosphate aqueous solution at pH 2.5, purified water, and 20% (w/v%) of an HSCD aqueous solution at the ratio of 2:1:1. A stock solution containing all four stereoisomers was prepared at the concentration of about 0.25 mg/mL by dissolving in methanol first and diluting with purified water. The final stock solution contained methanol and water at the ratio of 10/90 (v/v%). The stock enantiomer solution was further diluted with purified water serially to the concentration of about 1 μg/mL. The concentration at 1 μg/mL was the limit of quantitation solution. Sertraline hydrochloride (*cis*-(1*S*,4*S*) enantiomer, **1**) was dissolved in methanol first and diluted with purified water to the concentration of 1.0 mg/mL, and the volume ratio of methanol to water in sertraline hydrochloride sample solution was 10:90. An enantiomer mixture for the chiral selector study was prepared at the similar manner with the concentration range of 20–40 μg/mL.

2.3. Instrument method

All experiments were performed on a Hewlett-Packard ^{3D}CE instrument, model G1600AX, equipped with a UV diode array detector. Data were acquired at a rate of 2.5 Hz using Chemstation software, Rev. A.08.03. Fused silica capillaries (50 μm i.d. × 365 μm o.d.) from the same batch (Polymicro Technologies, Phoenix, AZ, USA) were utilized. A total length of 32 cm was used, and the distance to the detection window from the inlet (or injection end) of the capillary was 23.5 cm. The cassette holding the capillary column was controlled at the temperature of 12 °C during the course of method evaluation. The temperature of the autosampler was ambient. The absorbance was measured at three wavelengths: 200, 215, and 230 nm. The wavelength for quantitation and graphic illustration was 200 nm. The applied voltage was 10 kV with the reversed polarity setting (negative at the inlet or injection end). The voltage ramp time was 0.1 min. The samples were delivered into the capillary by applying a positive pressure of 25 or 15 mbar to the inlet (or cathode) for 5 s.

2.4. Column treatment

Fresh capillaries were pretreated by flushing with 50 mM triethylammonium phosphate buffer for 10 min, water for 5 min, and the running buffer for 10 min. This procedure was also used at the beginning of each sequence of runs,

or whenever the chiral selector was changed within the same sequence. Before the introduction of a sample, the capillary was flushed with buffer for 2 min. A fresh capillary was used whenever an experiment was performed on a different day.

3. Results and discussion

3.1. Method development rational

Sertraline is a basic compound. At pH 2.5, sertraline enantiomers are protonated. Highly sulfated cyclodextrins contain the negative charged sites at the outer and inner surface of the molecule. This provides the electrostatic interaction among sertraline stereoisomers and HSCDs in addition to the hydrogen bonding between the analytes and HSCDs as well as the cavity fitting. At pH 2.5, the electroosmotic flow is strongly suppressed. The negatively charged HSCDs migrate toward the anode end of the capillary [28], and the positively charged sertraline stereoisomers strongly interact with HSCDs and also migrate toward the anode. Depending upon the resolution and separation time of a specific application, the separation can be achieved either by delivering samples from the end with a longer distance to the detection window (normal migration direction) and reversing voltage polarity, or by delivering samples from the shorter end to the detection window and applying the voltage polarity conventionally. In our study, to maximizing the resolution in order to achieve adequate resolution for the enantiomeric impurities present at the trace level in bulk drug substance, we chose to deliver samples from the longer end to the detection window and reverse the voltage polarity.

3.2. Choice of voltage

To determine the proper voltage for the separation, an Ohm's law plot was generated. After the capillary was conditioned with each highly sulfated cyclodextrin at the room temperature, the voltage at 5, 7.5, 10, 12.5, and 15 kV was applied separately. The power per unit length was obtained by the Chemstation software. The Ohm's law plot was demonstrated in Fig. 2. The power at a given voltage for α-, β-, and γ-HSCD was the same. Usually, the applied voltage should be kept within the linear range. In our case, although the Ohm's law plot was not linear beyond 5 kV, our experiments demonstrated that the voltage could be extended to 10 kV. With the consideration of obtaining an efficient separation and controlling the Joule heating within a limited range, a voltage at 10 kV was used for the following studies.

3.3. Effect of the size of cyclodextrin cavity on separation

The three HSCDs, α, β, and γ, were added to the triethylammonium phosphate buffer separately. The mixtures of stereoisomers at the concentrations ranging from 20 to

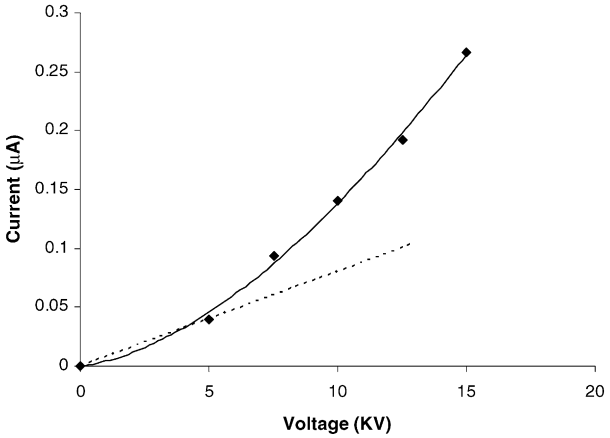

Fig. 2. Ohm's law plot at room temperature.

40 μg/mL were injected hydrodynamically. The resulting electropherograms are shown in Fig. 3. The three HSCDs demonstrated different selectivity towards the stereoisomers primarily due to the difference of cavity size and orientation of interaction between the analytes. The overall elution order of the four stereoisomers with β- and γ-HSCD in the running buffers was similar except that there was no resolution between *cis*-(1*R*,4*R*)-sertraline (**2**) and *trans*-(1*R*,4*S*)-sertraline (**4**) when HS-β-CD was used. More interestingly, the elution order of *cis*-(1*S*,4*S*)-sertraline (**1**) and *trans*-(1*S*,4*R*)-sertraline (**3**) changed when HS-α-CD was replaced with HS-γ-CD in the running buffer. The results of elution times and resolutions are presented in Table 2. The proposed explanation to this observation is shown in Figs. 4 and 5. When HS-α-CD was used as the pseudostationary phase, the dichlorophenyl moiety, the smaller end of the molecule, fit into α-CD cavity better, while HS-γ-CD was used as the pseudo stationary phase, the naphthalenamine moiety, a bigger end of the molecule, fit into γ-CD cavity better. This is supported by the molecular modeling software Hyper Chem that provides the detailed atomistic simulations. The cavity of α-CD fits better for molecules with one six-member ring and the cavity of γ-CD fits better for those with two six-member rings. As a result, a different selectivity for the stereoisomers was obtained. When HS-α-CD was used as

Table 2
Effect of cyclodextrin size on the separation

HS-α-CD				
Elution order	**1**	**2**	**4**	**3**
Retention time (min)	5.07	5.30	5.83	8.94
Resolution	NA	$R_{1,2}$ = 2.44	$R_{2,4}$ = 4.90	$R_{4,3}$ = 20.53
HS-β-CD				
Elution order	**3**	**2** and **4**	**2** and **4**	**1**
Retention time (min)	4.14	4.60	4.60	4.91
Resolution	NA	$R_{3,2}$ = 6.26	$R_{2,4}$ = 0	$R_{4,1}$ = 4.26
HS-γ-CD				
Elution order	**3**	**2**	**4**	**1**
Retention time (min)	3.57	4.12	4.36	5.09
Resolution	NA	$R_{3,2}$ = 10.94	$R_{2,4}$ = 4.17	$R_{4,1}$ = 9.64

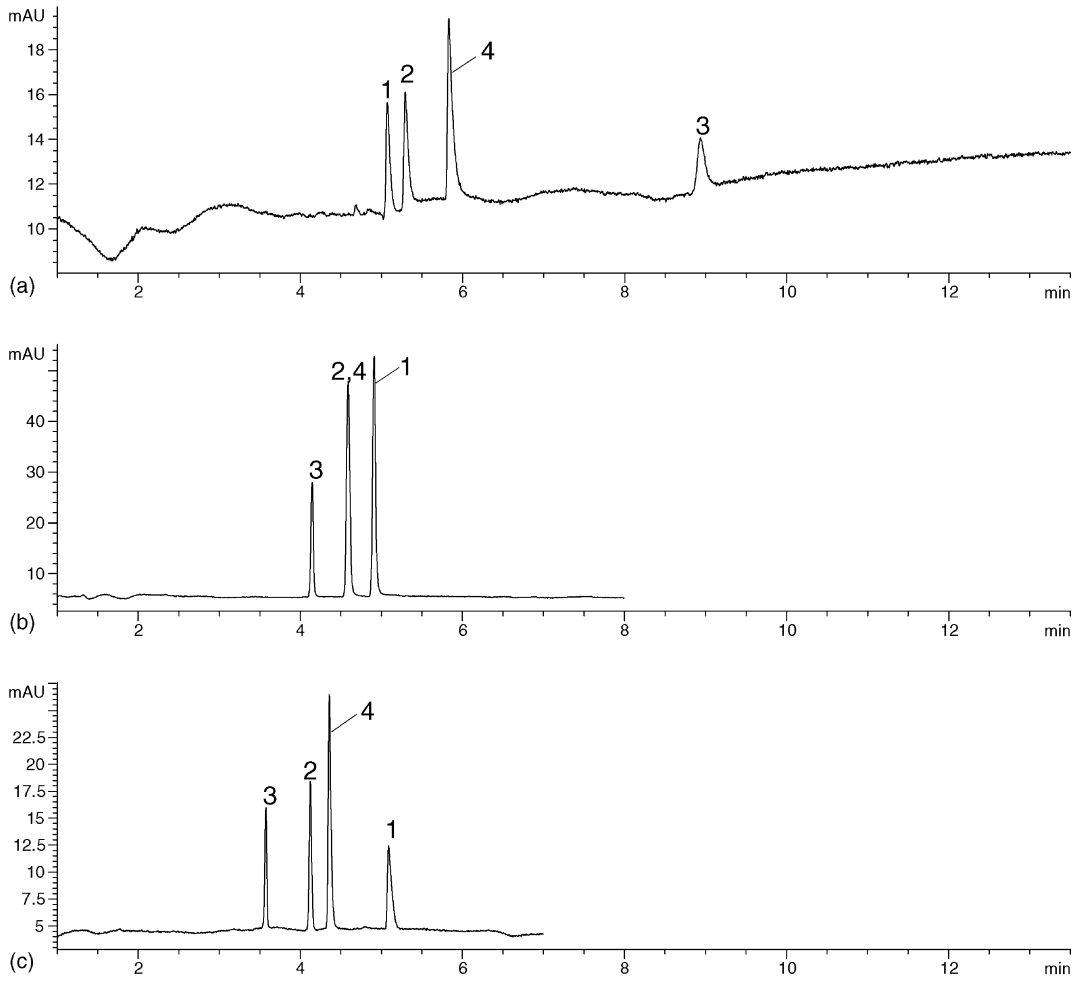

Fig. 3. Effect of chiral selector on separation. (Experimental conditions: Temperature was at 12 °C; stereoisomer concentrations were about 20–40 μg/mL; voltage was −10 kV; hydrodynamic injection was at pressure of 15 mbar for 4 s. The running buffer was 25 mM triethylammonium phosphate at pH 2.5 with 5% (w/v%) HS-α-CD in Fig. 5a; 5% (w/v%) HS-β-CD in Fig. 5b and 5% (w/v%) HS-γ-CD in Fig. 5c.)

the pseudostationary phase, the critical pair was between *cis*-(1*S*,4*S*) (**1**) and *cis*-(1*R*,4*R*) (**2**) stereoisomers with the resolution of 2.4 as shown in Fig. 3a and the active compound *cis*-(1*S*,4*S*) (**1**) stereoisomer was eluted first. When HS-γ-CD was used as the pseudostationary phase (Fig. 3c), the critical pair was between *cis*-(1*R*,4*R*) (**2**) and *trans*-(1*R*,4*S*) (**4**) stereoisomers with the resolution of 4.2 and the active *cis*-(1*S*,4*S*) (**1**) stereoisomer was eluted last. Therefore, use of HS-γ-CD as the chiral selector would result a superior analytical method with a substantial resolving power for the quantitation of trace amounts of sertraline stereoisomers in the bulk drug substance.

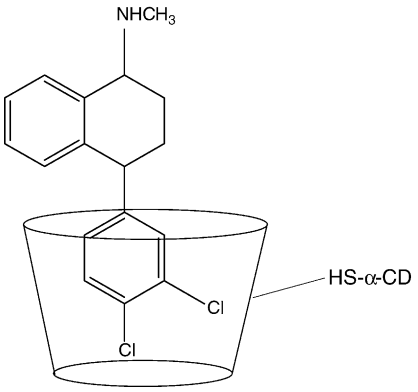

Fig. 4. Chiral recognition of HS-α-CD on sertraline.

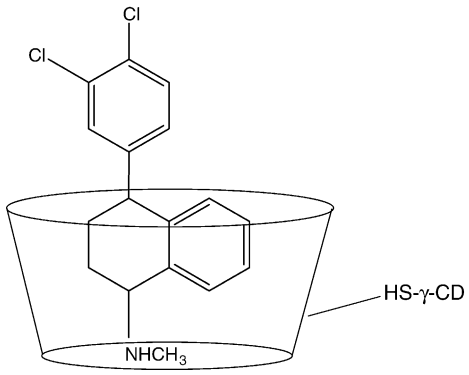

Fig. 5. Chiral recognition of HS-γ-CD on sertraline.

3.4. Method development

To obtain the optimized temperature, the text mixture at the concentration range from 20 to 40 μg/mL was tested at the controlled cassette temperature of 12, 18, 22, 25, 30, 40, 50, and 60 °C. Although the lowest setting of the instrument was nominally 5 °C, due to insufficient cooling, 12 °C was the lowest temperature that we have used. The highest critical resolution was found at 12 °C when the running buffer was 5% (w/v%) HS-γ-CD in 25 mM triethylammonium phosphate at pH 2.5 was used. When the temperature of the capillary was held at 12 °C, different concentrations of HS-γ-CD in w/w% such as 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, and 10.0 in 25 mM triethylammonium phosphate at pH 2.5 were tested. It was found that at the concentration range from 2.5 to 5.0%, the overall separation including peak shape and resolution was the best. Since the 50 mM phosphate buffer at pH 2.5 was purchased, in order to keep enough buffering capacity and maintain low EOF, we did not decrease the buffer concentration below 25 mM.

The concentrated stock test mixture in 90/10 water/methanol was diluted with 25 mM phosphate buffer, 25 mM phosphate buffer with 5% (w/w) HS-γ-CD, and water, respectively, to reach the testing concentration range from 20 to 40 μg/mL. The experimental results confirmed that there was no difference in the electropherograms with the different sample solutions for the final method. Therefore, water was used to dilute the stock sample solutions. During the study, we used the photo diode array detector and collected the UV spectra of the compounds as well as the electropherograms at 200, 215 and 230 nm to compare the signal-to-noise ratio. The results demonstrated that the best signal-to-noise ratios for all four compounds were obtained at 200 nm. However, we did observe a slight difference in λ_{max} among the four compounds. An explanation of this observation is that what was detected at a given time was the

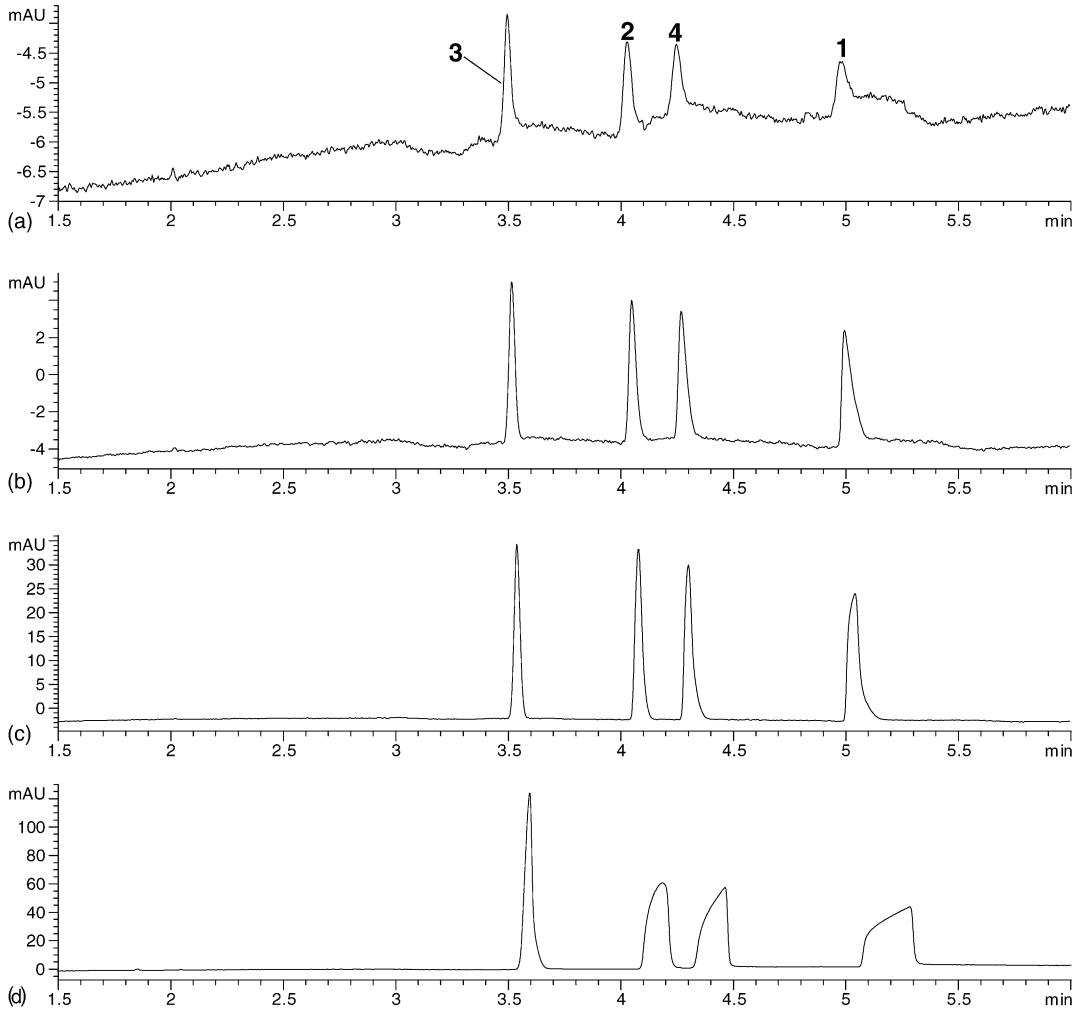

Fig. 6. Electropherograms of the linearity study. (Experimental conditions: $V = -10$ kV, hydrodynamic injection was at pressure of 25 mbar for 5 s. The running buffer was 5% (w/v%) HS-γ-CD in 25 mM triethylammonium phosphate at pH 2.5. Temperature = 12 °C. Concentrations of stereoisomer mixtures: (a) about 1.0 μg/mL, (b) about 10 μg/mL, (c) about 50 μg/mL, and (d) about 250 μg/mL.)

mixture of an individual compound and its complexes with HS-γ-CD. The λ_{max} of a given compound was shifted due to the presence of HS-γ-CD. It often occurred that worse experimental results were obtained if a capillary was stored for a certain time after being used. It seemed that the surface condition had been changed irreversibly. Therefore, it is recommend that a fresh capillary be used for an experiment performed on a different day. The experimental conditions used for the validation experiments below were as follows: the running buffer was 5% (w/v%) HS-γ-CD in 25 mM triethylammonium phosphate at pH 2.5, the temperature of the capillary cassette was held at 12 °C, the voltage was −10 kV, the data collection time per injection was not more than 10 min, the hydrodynamic injection pressure was 25 mbar for 5 s, and the UV wavelength for data collection was at 200 nm.

3.5. Limited validation of the optimized method

The validation demonstrated here only covered key elements of an analytical method. It is to show the feasibility of this method to be used for the routine analysis.

3.5.1. Linearity, limit of quantitation, and limit of detection

Mixtures of enantiomers at concentrations from 250 to 1 μg/mL were prepared. The resulting electropherograms are shown in Fig. 6. Although the peak shape was distorted for the later eluting Compounds **1**, **2**, and **4** at the concentration of 250 μg/mL, the linearity plot of concentration versus corrected area for all four compounds had a linear correlation coefficient (R^2) of not less than 0.999, and the intercept was not more than 0.007, as shown in Fig. 7a–d. The limit of quantitation of the stereoisomer impurities, **2**–**4**, was 1.0 μg/mL, shown in Fig. 6a that demonstrated a signal-to-noise ratio of about 10 for each compound, and the limit of detection of the stereoisomer impurities, **2**–**4**, was about 0.25 μg/mL for the given experimental conditions. A drug substance sample at the concentration of 1.0 mg/mL was prepared. The electropherogram in Fig. 8 showed that trace amounts of stereoisomer impurities **2**–**4** were present. The limit of quantitation at the concentration of 1.0 μg/mL was equivalent to 0.1% of the sample concentration. As there was an adequate resolution between each pair of stereoisomers, and especially, the parent compound eluted the last, a larger amount of injection of the drug substance sample would not interfere the quantitation of its stereoisomeric impurities **2**–**4**. Therefore, an

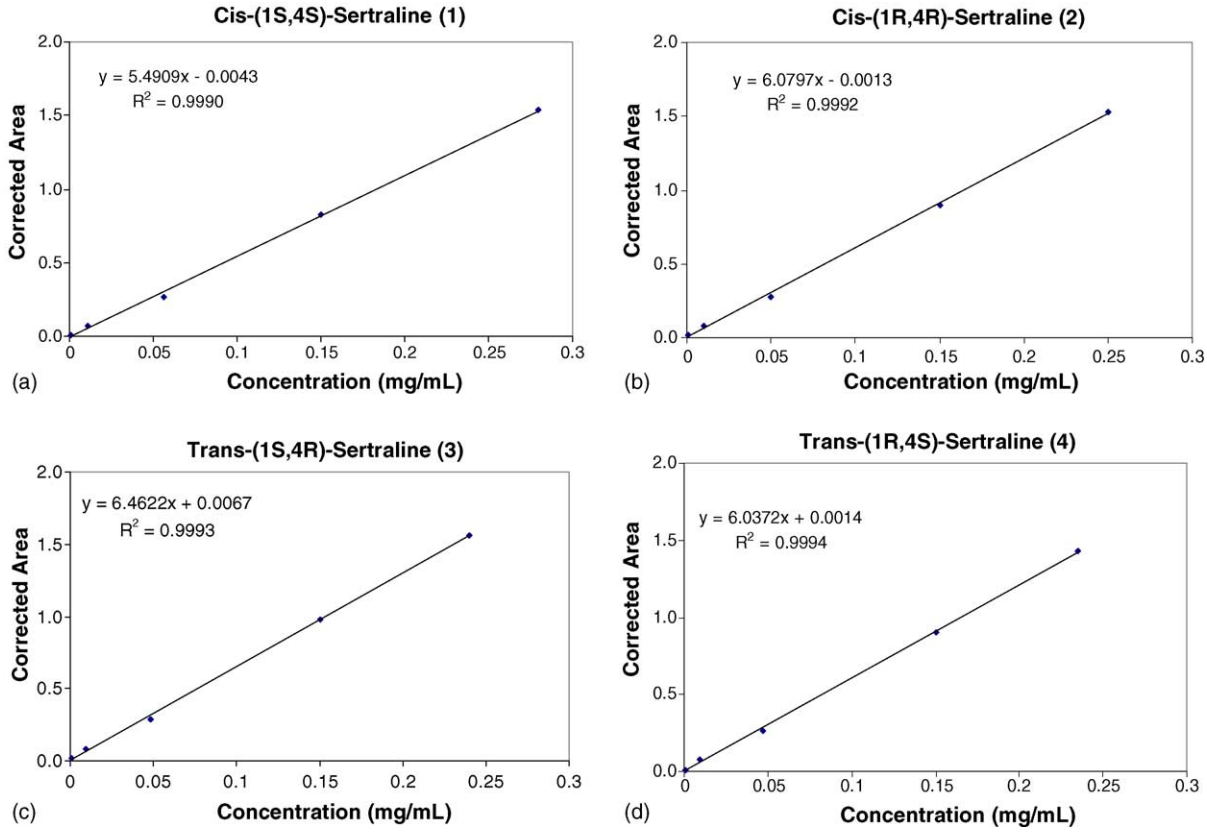

Fig. 7. (a) Linearity plots of: (a) *cis*-(1*S*,4*S*)-sertraline HCl (**1**); (b) *cis*-(1*R*,4*R*)-sertraline HCl (**2**); (c) *trans*-(1*S*,4*R*)-sertraline HCl (**3**); (d) *trans*-(1*R*,4*S*)-sertraline HCl (**4**).

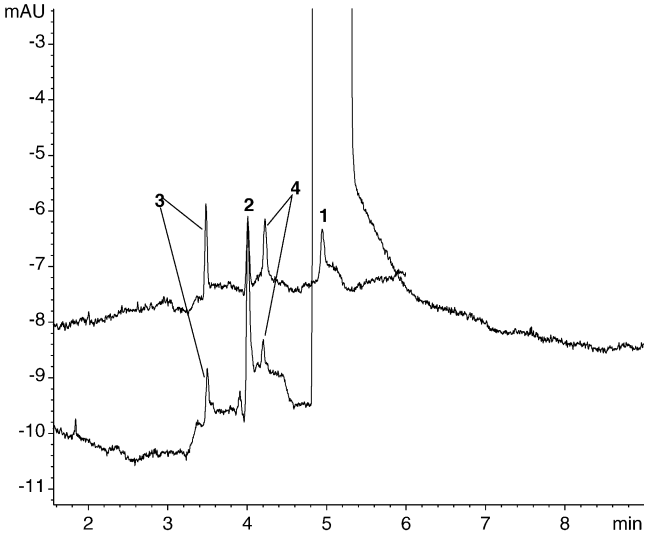

Fig. 8. Overlaid electropherograms of sertraline stereoisomer impurities at 1.0 μg/mL and sertraline hydrochloride drug substance at 1.0 mg/mL. Experimental conditions were the same as those in Fig. 6.

even lower quantitation and detection limit in percent of the active drug can be easily achieved by increasing the drug substance concentration. (*Note:* Practically, it is recommended that the linearity range of the impurities be evaluated in the presence of the main component at its sample concentration or 1 mg/mL in this case.)

3.5.2. Quantitative analysis

The ability of an analytical method to be able to identify and quantitate a known or an unknown compound is reflected by its accuracy and precision. The accuracy and precision of the method developed for the separation of the stereoisomeric impurities from the active sertraline hydrochloride stereoisomer (**1**) were demonstrated by injecting the stereoisomer mixtures at the concentrations ranging from 1.0 to 250 μg/mL in triplicate.

3.5.2.1. Amount of analyte obtained by using corrected peak area. The concentration of each stereoisomeric impurity found in each solution was obtained by using the external standard method and the results are represented in Table 3. Eq. (1) was used for the calculation of the amount of impurity in mg/mL.

$$\text{Concentration} = A_{\text{imp}} \times \frac{C_{\text{exstd}}}{A_{\text{exstd}}} \times \frac{1}{\text{RRF}} \quad (1)$$

where A_{imp} is the area of an impurity in sample solution, C_{exstd} the concentration of the external standard that contained 10 μg/mL of *cis*-(1*S*,4*S*)-sertraline (**1**), A_{exstd} the average peak area of the external standard, and RRF the relative response factor of an impurity. RRF is defined as:

$$\text{RRF} = \frac{\text{RF}_{\text{imp}}}{\text{RF}_{\text{std}}} \quad (2)$$

where RF_{imp} is the response factor of an impurity that is equal to the slope from the linearity plot for the impurity, and RF_{std} the response factor of the standard sertraline (**1**) that is equal to the slope from the linearity plot of sertraline (**1**). The relative response factors of the stereoisomeric impurities were found to be 1.1, 1.2, and 1.1 for stereoisomers **2**, **3**, and **4**, respectively. It was found that the amount of response factor was related to the interaction with the HSCD. The analytes migrated through the detection window in the forms of both by itself and HSCD–sertraline complexes. The portion of HSCD–sertraline complexes contributed to the difference

Table 3
The concentration of impurity found—external standard method

Compound no.	Solution no.	Concentration (mg/mL) Injection 1	Concentration (mg/mL) Injection 2	Concentration (mg/mL) Injection 3	Average	R.S.D. (%)
	1	0.191	0.230	0.208	0.210	9.2
	2	0.118	0.128	0.120	0.122	4.2
2	3	0.0445	0.0354	0.0331	0.0377	15.9 (4.6)[a]
	4	0.00986	0.0113	0.0103	0.0105	6.9
	5	0.00222	0.00208	0.00189	0.00207	8.0
					Average:	8.9 (6.6)[a]
	1	0.183	0.217	0.205	0.202	8.5
	2	0.122	0.142	0.124	0.129	8.6
3	3	0.0412	0.0359	0.0339	0.0349	10.3 (4.1)[a]
	4	0.0104	0.0120	0.0100	0.0108	9.6
	5	0.00284	0.00310	0.00245	0.00280	11.6
					Average:	9.7 (8.5)[a]
	1	0.182	0.215	0.192	0.196	8.5
	2	0.117	0.129	0.124	0.123	5.0
4	3	0.0433	0.0341	0.0315	0.0363	17.3 (5.6)[a]
	4	0.0098	0.0108	0.0096	0.0100	6.3
	5	0.00174	0.00177	0.00164	0.00172	4.1
					Average:	8.2 (5.9)[a]

Note: The experimental conditions were the same as those in Fig. 6 except the concentration of the solution.

[a] Result in parenthesis obtained when the outlier (Injection no. 1 of Solution no. 3) was excluded.

Table 4
The concentration of impurity found—internal standard method

Compound no.	Solution no.	Concentration (mg/mL) Injection 1	Concentration (mg/mL) Injection 2	Concentration (mg/mL) Injection 3	Average	R.S.D. (%)
	1	0.248	0.249	0.255	0.251	1.4
	2	0.146	0.149	0.144	0.146	1.7
2	3	0.0510	0.0515	0.0523	0.057	1.1
	4	0.00996	0.01056	0.01094	0.012	4.2
	5	0.00163	0.00150	0.00157	0.0017	3.7
					Average:	2.4
	1	0.238	0.236	0.250	0.285	2.7
	2	0.151	0.166	0.148	0.177	5.3
3	3	0.0473	0.0522	0.0534	0.060	5.4
	4	0.0105	0.0112	0.0106	0.013	2.9
	5	0.00209	0.00223	0.00203	0.0025	4.1
					Average:	4.1
	1	0.237	0.233	0.235	0.261	0.7
	2	0.144	0.151	0.148	0.162	1.9
4	3	0.0496	0.0497	0.0497	0.055	0.1
	4	0.0099	0.0101	0.0102	0.011	1.2
	5	0.00128	0.00128	0.00136	0.0014	3.2
					Average:	1.4

Note: The experimental conditions were the same as those in Fig. 6 except the concentration of the solution.

of the response factor among the four stereoisomers. It is clear that the relative standard deviation (%) of the amount of an impurity found from the injection of the same sample solution three times was quite high. This poor precision may be due to the poor consistency of the sample delivery system and the fluctuation of EOF. Based upon the result of *Q*-test, the Injection 1 of Solution no. 3 was an outlier and it was the cause of the high relative standard deviation. This variation of the amount of sample delivered to the capillary can be corrected by using the internal standard method for quantitation. We used the parent compound sertraline HCl (**1**) as the internal standard just for the sake of demonstration. Practically, another compound that elutes away from the group of stereoisomers could also be used as the internal standard. The amount of the impurities in each solution was recalculated by using Eq. (3) and the data are presented in Table 4.

$$\text{Concentration} = A_{\text{imp}} \times \frac{C_{\text{instd}}}{A_{\text{instd}}} \times \frac{1}{\text{RRF}} \quad (3)$$

where A_{instd} is the area of the internal standard in sample solution, and C_{instd} the concentration of the internal standard in sample solution. The results in Table 4 demonstrated the overall improvement of the precision reflected by the relative standard deviation (%). Therefore, the use of an internal standard for the quantitation is necessary to obtain an acceptable accuracy and precision. Furthermore, data in both Tables 3 and 4 demonstrated that the average relative standard deviation (%) decreased with the elution order such as **3**, **2**, and **4**. The closer was a compound to the referenced standard compound, the lower the relative standard deviation. This was quite possibly due to the fluctuation of electroosmotic flow within a run, so that the further a compound eluted from the reference compound, the worse the precision and accuracy for that compound.

3.5.2.2. Retention time. Retention time data for each compound are given in Table 5. Although the relative standard deviation (%) of a compound obtained from the injections of the same solution three times was less than 2.0%, the overall relative standard deviations (%) of Compounds **4** and **1**, the last two eluting peaks, obtained by including all sample solutions and all injections, were at or greater than 2.0%. This is due to the large variations in retention times of the compounds in the most concentrated sample solution at about 250 μg/mL. Such amount of variation in retention time may introduce difficulty in peak identification. Excluding the data points at the highest concentration for the last three eluting peaks, Compounds **1**, **2**, and **4**, the overall relative standard deviations (%) of not more than 1.0% for all analytes were obtained. Therefore, for the purpose of tentative identification via retention times, the method is only valid to the concentration level up to about 150 μg/mL based upon this study.

3.5.2.3. Theoretical plate number. In pharmaceutical industry, in addition to the commonly used parameters such as the relative standard deviation of peak area, retention time, and resolution, another criterion often used to measure the system suitability for quantitative analysis is the theoretical plate number, which is guided by the commonly referred United State Pharmacopia [30]. The number of theoretical plates (n) of each peak in each injection demonstrated in Table 6 was obtained by the tangent method:

$$n = 16 \times \left(\frac{t_i}{W_{\text{B}}}\right)^2 \quad (4)$$

where t_{i} is the retention time of a peak i in min and W_{B} the base width of the peak in min, obtained by intersecting tangents through the inflection points with the baseline. The numbers less than 10,000 in bold in Table 6 were from the

Table 5
Precision–retention time

Compound no.	Concentration (mg/mL)	Ratio of molarity[a]	RT (min) Injection 1	RT (min) Injection 2	RT (min) Injection 3	Average	R.S.D. (%)
1	0.28	0.052	5.28	5.29	5.23	5.27	0.6
	0.17	0.031	4.96	4.90	4.92	4.93	0.7
	0.056	0.010	5.04	5.01	5.00	5.02	0.5
	0.011	0.002	5.00	4.98	4.96	4.98	0.3
	0.0011	0.0002	4.97	4.96	4.95	4.96	0.3
					Average	5.03	2.7
					Average[b]	4.97	0.8
2	0.25	0.047	4.18	4.16	4.15	4.16	0.4
	0.15	0.028	4.12	4.12	4.11	4.12	0.1
	0.05	0.009	4.08	4.07	4.06	4.07	0.2
	0.010	0.002	4.05	4.04	4.03	4.04	0.3
	0.0010	0.0002	4.03	4.02	4.01	4.02	0.2
					Average	4.08	1.4
					Average[b]	4.06	1.0
3	0.24	0.045	3.60	3.53	3.57	3.56	0.9
	0.14	0.027	3.55	3.54	3.53	3.54	0.3
	0.048	0.009	3.54	3.53	3.53	3.53	0.1
	0.010	0.002	3.52	3.51	3.50	3.51	0.2
	0.0010	0.0002	3.50	3.49	3.48	3.49	0.2
					Average	3.53	0.8
4	0.24	0.044	4.46	4.46	4.42	4.45	0.5
	0.14	0.026	4.28	4.24	4.25	4.26	0.5
	0.047	0.009	4.30	4.28	4.28	4.29	0.3
	0.009	0.002	4.27	4.26	4.25	4.26	0.3
	0.0009	0.0002	4.25	4.24	4.23	4.24	0.3
					Average	4.30	2.0
					Average[b]	4.26	0.5

Note: The experimental conditions were the same as those in Fig. 6 except the concentration of the solution.
[a] Ratio of molarity is defined as the molarity of analyte to that of HS-γ-CD.
[b] The data point at the highest concentration was not included.

Table 6
Theoretical plate number

Compound no.	Concentration (mg/mL)	Ratio of molarity[a]	Plate no. Injection 1	Plate no. Injection 2	Plate no. Injection 3
1	0.28	0.052	**3700**	**2900**	**3500**
	0.17	0.031	**9100**	**4100**	**5500**
	0.056	0.010	44900	67600	69300
	0.011	0.002	48600	42700	44600
	0.0011	0.0002	39800	49200	52800
2	0.25	0.047	**49000**	**6400**	**8000**
	0.15	0.028	26500	23200	21000
	0.05	0.009	63400	78600	76400
	0.010	0.002	64200	72161	53200
	0.0010	0.0002	49100	57500	58900
3	0.24	0.045	48100	40900	45300
	0.14	0.027	62800	33000	41400
	0.048	0.009	57300	54100	65800
	0.010	0.002	65300	65000	59400
	0.0010	0.0002	50300	44600	52700
4	0.24	0.044	**8400**	**6900**	**8000**
	0.14	0.026	27100	18500	25900
	0.047	0.009	74300	81800	81600
	0.009	0.002	66700	64500	66000
	0.001	0.0002	57500	50400	55500

Note: The experimental conditions were the same as those in Fig. 6 except the concentration of the solution.
[a] Ratio of molarity is defined as the molarity of analyte to that of HS-γ-CD.

distorted peaks of Compounds **1**, **2**, and **4** in the sample solution at about 250 μg/mL and **1** in the sample solution at about 150 μg/mL. Therefore, the number of theoretical plates also confirms that although the method is linear up to the concentration of about 250 μg/mL, for the purpose of quantitation of stereoisomer impurities, the range of the method is from 1 to 150 μg/mL, which is about two orders of magnitude.

4. Conclusion

A cost effective analytical method for the separation of sertraline enantiomers using capillary electrophoresis was developed by using HS-γ-CD as the chiral selector. This method demonstrated superior enantiomeric selectivity and resolution to the previously published one [29]. A limit of quantitation of each enantiomeric impurities at 0.1% or lower of the drug substance sample concentration can be easily obtained. In this study, we found that depending upon the chemical structure of the analyte, the different size of cyclodextrin might provide an adverse effect on the selectivity and elution order. In addition, for the purpose of quantitation, an internal standard is necessary for this CE method. Furthermore, for the purpose of identification and quantitation, this CE method has a working range of about two orders of magnitude.

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