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Comparing cyclodextrin derivatives as chiral selectors for enantiomeric separation in capillary electrophoresis

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Abstract

A total of 26 different cyclodextrin (CD) derivatives with different functional groups and degrees of substitution were tested against 35 basic pharmaceutical compounds in an effort to investigate their effectiveness as chiral selectors for enantiomeric separation in capillary electrophoresis (CE). Testing was performed under the same conditions using a low pH buffer (25 m*M* phosphate buffer at pH~2.5). Five CD derivatives, namely, highly sulfated-β-CD, highly sulfated-α-CD, hydroxypropyl-β-CD (degree of substitution~1), heptakis-(2,6-*O*-dimethyl)-β-CD, and heptakis(2,3,6-*O*-trimethyl)-β-CD were identified to be most effective for enantiomeric separations and have a wide range of enantiomeric selectivity towards the model compounds. Over 90% of the model compounds were enantiomerically resolved with the five identified CD derivatives, at a minimum resolution of 0.5. An additional 20 compounds were also tested to demonstrate the validity of the identified CD derivatives. The five CD derivatives were recommended as the starting chiral selectors in developing enantiomeric separation methods by CE.
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1. Introduction

More pharmaceutical products are being developed as single enantiomer drugs as differences in pharmacologic and toxicologic properties of the enantiomers of chiral drugs are recognized [1–3]. Enantiomeric purity of pharmaceutical compounds has become an important quality issue in pharmaceutical development. Analytical chemists in pharmaceutical industries are under increasing pressure to significantly reduce method development time, and moreover the methods with short analysis time are often required. Capillary electrophoresis (CE) has been increasingly selected as the technique of choice for enantiomeric separations for its high efficiencies and short analysis time [3–8]. Enantiomeric resolution in CE occurs through differential interactions of enantiomers with chiral selectors added to the running buffers [9]. Due to the capillary dimensions required for CE operation, only a very small quantity of the chiral selectors is needed, thereby reducing the operation costs. Another advantage of using CE for

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enantiomeric separations is the fact that different selectivity can be obtained by simply changing the type of chiral selectors added to the running buffers without changing capillaries, therefore allowing an automated approach to method development.

The most commonly used chiral selectors in CE are cyclodextrins and various derivatives [10–14]. Native cyclodextrins (CDs) are cyclic oligosaccharides consisting of glucopyranose units with the shape of a truncated cone [15]. Chemical modification adds functional groups (e.g., hydroxypropyl, methyl, sulfobutyl-ether, and sulfate groups) to native CDs, which results in significant changes in physicochemical property such as solubility [16]. A common approach to method development in pharmaceutical analysis is to select a small number of CD derivatives for initial testing in an effort to find the CD derivative that provides desired enantiomeric separation for the target compound [8]. If the initial CDs fail to provide desired resolution, more CDs need to be tested. Once the right CD derivative is identified, other running parameters (e.g., CD concentration, buffer pH, ionic strength, applied voltage, etc.) are optimized to finalize the method. Obviously, judicial choice of CD derivatives is critical to the success of this approach and reducing method development time. On one hand, the large number of CD derivatives with various functional groups and degree of substitution provides method development chemists with an ample choice of chiral selectors. On the other hand, finding the right CD with desired selectivity may not be straightforward and may take a significant amount of development time.

The purpose of this study was to identify the CD derivatives based on their selectivity towards pharmaceutical compounds, which can provide good chances (>80%) to achieve desired enantiomeric separation in only a few trial runs. In practice, these CD derivatives may serve as the initial chiral selectors in developing enantiomeric methods; thus method development time is reduced by avoiding unnecessary testing of many CD derivatives. Fillet et al. recommended five CD derivatives, heptakis (2,6-di-*O*-methyl)-β-CD, heptakis (2,3,6-tri-*O*-methyl)-β-CD, hydroxypropyl-β-CD, carboxymethyl-β-CD, and sulfobutyl-β-CD for method development based on their study on 30 pharmaceutical compounds [17]. Sulfated CD derivatives were used in another method development strategy developed by Zhou et al. [18]. Lin et al. reported enantiomeric separation of 123 drug compounds using seven native and neutral CD derivatives [19]. Recently, Phinney et al. investigated the effect of five neutral and anionic CD derivatives on the enantiomeric separation of 14 drug compounds [20]. However, the literature studies on the effect of CD derivatives on the enantiomeric separation of pharmaceutical compounds were limited by either the number or type of CD derivatives or the number of compounds. The current study complimented the literature reports by including both a significant number of CD derivatives with various functional groups and degree of substitution and a large number of pharmaceutical compounds in the study design.

In this study, a total of 26 commercially available CD derivatives with different functional groups, degree of substitution and from different sources were tested using a pool of 55 pharmaceutical compounds including both marketed drugs and proprietary compounds. The effectiveness of CD derivatives was evaluated based on their abilities to enantiomerically resolve the model compounds. This paper summarizes the findings and experimental data from over 1000 CE experiments involved in this study.

2. Experimental

2.1. Instrumentation

All CE experiments were performed on a Beckman P/ACE MDQ CE system equipped with a diode array detector (Beckman Coulter, Fullerton, CA, USA). The migration of model compounds was monitored at 200 nm. Uncoated fused-silica capillaries from Polymicro Technologies (Phoenix, AZ, USA) were used for enantiomeric separations. The dimensions of the capillary were 40 cm (30 cm to the detector)×50 I.D.×375 μm O.D. Data acquisition and collection were performed by P/ACE Station Software (Version 2.0) from Beckman.

2.2. Cyclodextrins

All cyclodextrin derivatives were purchased from

Table 1
Cyclodextrin derivatives

Neutral CDs			Anionic CDs		
CD name	Vendor	D.S.	CD name	Vendor	D.S.
2-Hydroxypropyl-α-CD	Aldrich	0.52	Carboxymethyl-β-CD	Fluka	–
Hydroxypropyl-β-CD	Aldrich	0.95	Sulfobutyl-ether-β-CD	Cydex	0.6
Hydroxypropyl-β-CD	Aldrich	0.64	Sulfobutyl-ether-β-CD	Cydex	1.0
Hydroxypropyl-γ-CD	Aldrich	0.6	Sulfated α-CD, sodium	Aldrich	1–1.6
2-Hydroxypropyl-α-CD	Fluka	0.6	Sulfated β-CD, sodium	Aldrich	1–1.6
2-Hydroxypropyl-β-CD	Fluka	0.6	Highly sulfated-α-CD	Beckman	1.8
2-Hydroxypropyl-γ-CD	Fluka	0.7	Highly sulfated-β-CD	Beckman	1.7
2-Hydroxypropyl-β-CD	Sigma	0.6	Highly sulfated-γ-CD	Beckman	1.6
2-Hydroxypropyl-β-CD	Sigma	0.7	Heptakis-6-sulfo-β-CD	Regis	1.0
Hydroxyethyl-β-CD	Aldrich	0.7	Heptakis(2,3-diacetyl-6-sulfo)-β-CD	Regis	3.0
2-Hydroxyethyl-β-CD	Fluka	0.7	Heptakis(2,3-dimethyl-6-sulfo)-β-CD	Regis	3.0
Methyl-β-CD	Aldrich	1.6–2.0			
Methyl-β-CD	Fluka	1.6–2.0			
Heptakis-(2,6-*O*-dimethyl)-β-CD	Fluka	2.0			
Heptakis(2,3,6-*O*-trimethyl)-β-CD	Fluka	3.0			

various commercial sources. Table 1 lists the CD derivatives used in this study including chemical name, vendor and degree of substitution (D.S.). The names used by the vendors are cited without change for all the CD derivatives. The degree of substitution is defined as the average number of functional groups on each glucopyranose unit of the CD ($0 < D.S. < 3$). The D.S. for each CD was obtained from the certificate of analysis from the CD suppliers.

2.3. Model compounds

In the initial testing, 35 model compounds were selected including 19 commercial drug compounds and 16 investigational drug compounds internal of Johnson and Johnson Pharmaceutical Research and Development. The commercial drugs were racemic mixtures, including atenolol, bupivacaine, bupropion, chloroquine diphosphate, disopyramide, doxylamine, econazole, epinephrine, fluoxetine, miconazole, norephedrine, norepinephrine, ofloxacin, pindolol, propranolol hydrochloride, terbutaline, tramadol, trimipramine, and verapamil hydrochloride from Aldrich, Sigma and Fluka. The names and structures of the internal compounds are not shown for proprietary reasons.

In the confirmatory testing, 20 investigational drug compounds from internal sources were used to verify the results of the initial testing. These compounds were different from the 16 internal compounds used in the initial testing, and were labeled as compounds RWJ-1 to RWJ-10 and compounds JRF-1 to JRF-10. The prefix (RWJ or JRF) indicated the source of the compounds. The structures of RWJ compounds and JRF compounds were not disclosed for proprietary reasons.

The commercial compounds were prepared in a sample solvent of acetonitrile–water (50:50, v/v) at a concentration of 0.5–1 mg/ml. For the internal compounds, a sample solvent of acetonitrile–water (80:20, v/v) was used to prepare the sample solutions at a concentration of 0.3–1 mg/ml.

2.4. Running buffers

The running buffer used in both initial and confirmatory testing was 25 m*M* phosphate with pH adjusted to 2.5. An appropriate amount of each CD derivative was weighed and dissolved in the phosphate buffer to obtain a final concentration of 15 m*M*. The molar concentration was calculated based on the average molecular mass obtained from the vendor or calculated based on the average D.S. For highly sulfated CD solutions (20%, w/v) from Beckman, a dilution procedure was used. One part of the highly sulfated CD solutions was mixed with one part of water and two parts of 50 m*M* triethylammonium phosphate buffer (Beckman).

2.5. Experimental conditions

At the beginning of each day, a rinsing procedure was performed by washing the capillary with the following solutions in series at 20 p.s.i., 1 *M* NaOH (Beckman-Coulter) for 1 min, deionized water (Milli-Q gradient, Millipore) for 1 min, capillary conditioning solution (Beckman-Coulter) for 2 min, deionized water for 1 min, and the running buffer for 1 min (1 p.s.i.=6894.76 Pa). The enantiomeric separations were carried out at 20 kV if neutral CD derivatives were used, and at −15 kV if anionic CD derivatives were used. The capillary was installed in a cartridge thermostated at 15 °C, and the sample tray was set at 4 °C. Sample injection was made by applying a pressure of 0.1 p.s.i. at the injection end for 2 to 15 s depending on sample concentrations. The capillary was also rinsed with deionized water and the running buffer for 1 min each between runs. In the initial screening study, the separation was allowed to proceed for 15 min to reduce the total experiment time, and the analytes were pushed out of the capillary with a pressure of 20 p.s.i. for 2 min if not eluted within 15 min. In confirmatory testing, the separation was run for 30 min to allow slowly migrating analytes to be detected.

3. Results and discussion

3.1. Study rationale

This study was designed to evaluate the effectiveness of commercially available CD derivatives for enantiomeric separation of pharmaceutical compounds. In this study, 26 CD derivatives (15 neutral and 11 anionic) represented those commonly used in enantiomeric separations. The CD derivatives had various sizes (α, β, and γ), functional groups (hydroxypropyl, methyl, carboxymethyl, sulfobutylether, and sulfate groups), different degrees of substitution, and were from different vendors (Table 1). For the results to be meaningful and have practical values, selecting appropriate model compounds was critical in this study. The ideal compounds should be drug related and structurally diversified. In addition, the number of the model compounds should not be too small. To this end, a total of 55 model compounds were used in the current study, including 19 commercial drugs and 36 investigational drug compounds from internal sources. Most of these internal compounds were either at discovery or early development stage and presented special challenges to the study design since there was very limited information available on their solubility and chemical composition.

The current study was conducted in two phases, initial and confirmatory testing. The experimental plan for the initial testing was to run all the CD derivatives using 35 model compounds (19 commercial and 16 internal) under defined conditions and identify the CD derivatives that showed wide selectivity towards the model compounds. Considering the limitation in the number of model compounds and experimental conditions (e.g., CD concentration, buffer pH, etc.), it was necessary to test the identified CDs using a different set of model compounds to confirm the finding of the initial testing. In the second confirmatory study, four identified CD derivatives were tested under the same conditions using another set of 20 internal compounds.

The experimental conditions (e.g., CD concentration, buffer pH, ionic strength, voltage, and run time) were chosen based on literature reports and our own experience [17]. The CD derivatives were tested at only one concentration because first, the selected CD concentration was typical in method development. Secondly, it took over 900 runs to finish testing all the 26 CD derivatives against 35 model compounds at one CD concentration; and the time needed to test more CD concentrations would be impractical. It was fully realized that the CD concentration had significant effects on their resolving power. The results based on only one CD concentration may be limited; however, they were sufficient for the purpose set forth at the beginning of the study. The other running conditions were set up based on the fact that all the model compounds were basic in this study, and a low pH condition (pH~2.5) at moderate ionic strength (25 m*M*) was common for basic compounds.

The effectiveness of the CD derivatives as chiral selectors for enantiomeric separations was evaluated by calculating the percentage of model compounds that were resolved at a minimum resolution (R_s) of 0.5 by each CD derivative. This percentage was

referred as success rate in the study. Zhou et al. employed similar criteria in their studies to evaluate seven cyclodextrins [18]. Even though baseline separation was not achieved at R_s~0.5, it clearly demonstrated the potential to achieve better separations by optimizing other parameters, such as CD concentration, buffer pH, ionic strength. In this study, the CE run time was not adjusted for each CD in order to elute all model compounds. Instead, a fixed run time was used in all experiments to run the experiments in an automated fashion (15 min for the initial and 30 min for the confirmatory testing) in a reasonable time frame. One complication was that some compounds did not elute within the set run time. Those cases were counted as failures for the CD derivative tested, even though the CD may have separated the model compound at a later time. This treatment might be unfair to some CD derivatives, but it was impractical to wait for all the model compounds to migrate out of the capillary in a reasonable time.

3.2. Results of initial testing

3.2.1. Neutral CD derivatives

There are only two types of neutral CD derivatives that are commonly used, namely, hydroxypropylated and methylated CDs. However, different degrees of substitution and suppliers can complicate the selection of proper CD derivatives for method development. Nine hydroxypropylated CDs with various degrees of substitution from different suppliers were tested against 35 model compounds in this study, including two hydroxypropyl-α-CDs (HP-α-CD), two hydroxypropyl-γ-CDs (HP-γ-CD) from Aldrich and Fluka, and five hydroxypropyl-β-CDs (HP-β-CD) from Aldrich, Sigma, and Fluka (Table 1). In addition, two hydroxyethyl-β-CDs (HE-β-CD) from Aldrich and Fluka were also tested.

The bar charts in Fig. 1 represent the success rates of five HP-β-CDs tested in this study, and Fig. 2 shows the success rate of HP-α-CDs, HP-γ-CDs, and HE-β-CDs. HP-β-CD from Aldrich (D.S.~0.95) yielded the highest success rate of 42%; however, another HP-β-CD also from Aldrich (D.S.~0.64) gave a much lower success rate of 26%, possibly due to the lower degree of substitution (Fig. 1). In general, all HP-β-CDs outperformed HP-α-CDs and

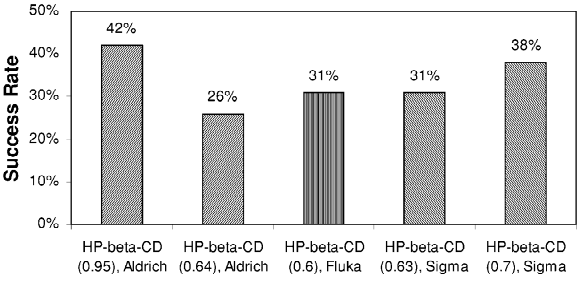

Fig. 1. Success rates of six hydroxypropyl-β-CDs (HP-β-CDs). The number in parentheses represents the degree of substitution of each CD derivative.

HP-γ-CDs by showing wider selectivity towards the model compounds. In addition, two HE-β-CDs showed lower success rates than HP-β-CDs with similar D.S.s, indicating the influence of the functional groups (i.e., hydroxypropyl vs. hydroxyethyl group) on the enantiomeric separation (Fig. 2). HP-γ-CDs yielded the lowest success rates of 10%; however, their selectivity was generally complementary to that of HP-β-CDs or HP-α-CDs. It is worth mentioning that the difference of the CD derivatives with the same D.S. from different sources was also noticed. For example, the HE-β-CD from Fluka (19%) gave a higher success rate than that from Aldrich (9%), and the HP-α-CD from Aldrich (26%) provided a higher success rate than the one from Fluka (18%). This demonstrated that the source of the CD derivative was also important in addition to the D.S. The experimental results on hydroxypropyl-CD derivatives indicated that the selectivity was not only dependent on the type of cyclodextrins (α-, β-,

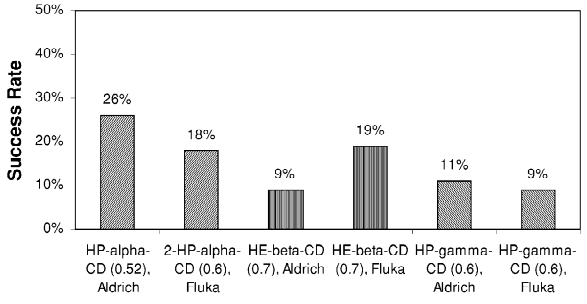

Fig. 2. Success rates of two hydroxypropyl-α-CDs (HP-α-CDs), two hydroxyethyl-β-CDs (HP-β-CDs), and two hydroxypropyl-γ-CDs (HP-γ-CDs). The number in parentheses represents the degree of substitution of each CD derivative.

or γ-CDs), but also on degrees of substitution and the sources of CD derivatives.

The hydroxy groups at the rims of cyclodextrins can also be modified with methyl groups in either a random or a controlled manner. Two randomly methylated CDs (methyl-β-CDs) with the same D.S. (1.6~2.0), but from different suppliers (Aldrich and Fluka) were selected. In addition, heptakis-(2,6-*O*-dimethyl)-β-CD (DM-β-CD) and heptakis (2,3,6-*O*-trimethyl)-β-CD (TM-β-CD) from Fluka with controlled substitution were included in the testing. The success rate of each methylated CD is shown in Fig. 3. DM-β-CD and TM-β-CD showed very similar success rates (29 vs. 30%) for the model compounds. Upon further examination of the data, however, it was noticed that the model compounds that each CD derivative resolved were quite different, indicating complementary selectivity of the two methylated CD derivatives. On the other hand, the randomly methylated β-CDs yielded lower success rates than DM-β-CD or TM-β-CD. This could be attributed to the slightly lower D.S. or the difference in the methylation position (i.e., C-2 and C-6 vs. C-3 hydroxyl) of the randomly methylated CDs.

3.2.2. Anionic CD derivatives

Native cyclodextrins can also be derivatized with ionizable functional groups, such as sulfate, carboxymethyl, and amino-alkyl groups to impart charges to the CD derivatives in the running buffers. Only anionic CD derivatives including sulfated CD derivatives, sulfobutyl-ether-β-CD, and carboxymethyl-β-CD were tested in this study since most of pharmaceutical compounds were basic. The negatively charged CD derivatives migrated towards the cathode under the experimental conditions (pH~2.5); therefore, the separation was performed with reversed polarity for all the anionic CD derivatives except for carboxymethyl-β-CD, which was neutral under current conditions.

Sulfated CD derivatives are modified with sulfate groups at the C-2, C-3 or C-6 hydroxyl positions. Two types of sulfated CD derivatives are commercially available, highly sulfated CDs (HS-CD) and single isomer sulfated CDs. Highly sulfated CDs from Beckman contain an average of 11, 12 and 13 sulfate groups for each HS-α-CD, HS-β-CD and HS-γ-CD molecule, respectively [21]. All sulfate groups are attached to the CD molecule at the C-2 and C-6 position exclusively, leaving the C-3 hydroxyl intact. The HS-α-CD and HS-β-CD from Aldrich are sodium salts and have 7–11 sulfate groups per CD molecule on average. Fig. 4 shows the success rates for this group of HS-CDs. In general, highly sulfated CDs provided much higher success rates than neutral CD derivatives. Over half of the model compounds were resolved by the three Beckman HS-CDs, with Beckman's HS-β-CD yielding the highest success rate of 68%. Some differences in the selectivity among the three Beckman HS-CDs were also observed, possibly due to different cavity sizes. For example, HS-β-CD gave bupivacaine much better separation than HS-γ-CD, as shown in Fig. 5. In comparison, the HS-CDs from Aldrich had lower success rates than those from Beckman, and a large difference was observed for the HS-α-CDs from Aldrich and Beckman (34 vs. 62%). It should be pointed out that HS-CDs (HS-α-CD and HS-β-CD) from Aldrich were tested at 15 m*M*, while the concentration of HS-α-CD and HS-β-CD from Beckman was estimated to be around 20

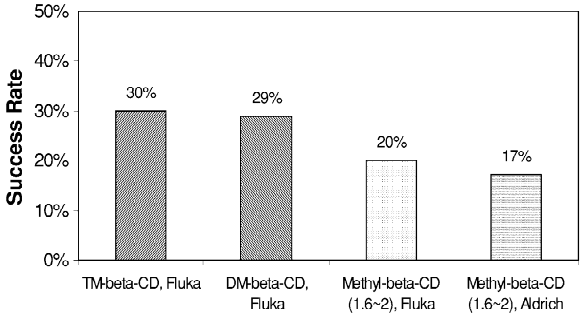

Fig. 3. Success rates of different methylated-β-CD derivatives.

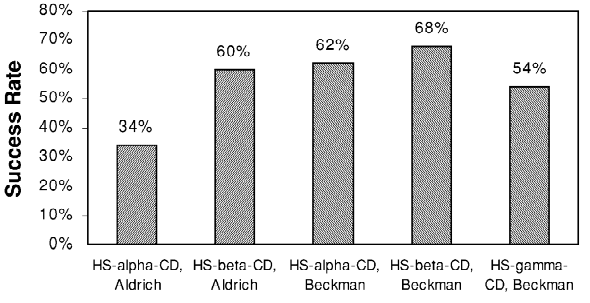

Fig. 4. Success rates of five highly sulfated CD derivatives.

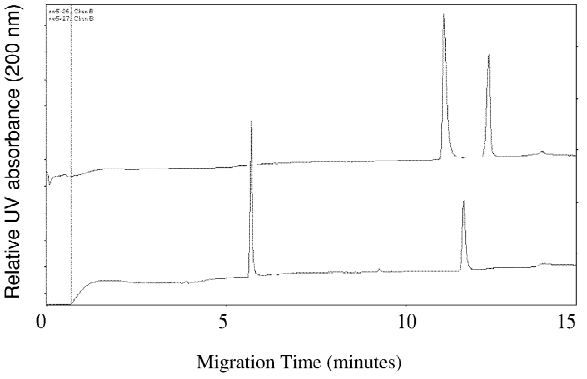

Fig. 5. Enantiomeric separation of bupivacaine racemate with Beckman's HS-β-CD (bottom) and HS-γ-CD (top).

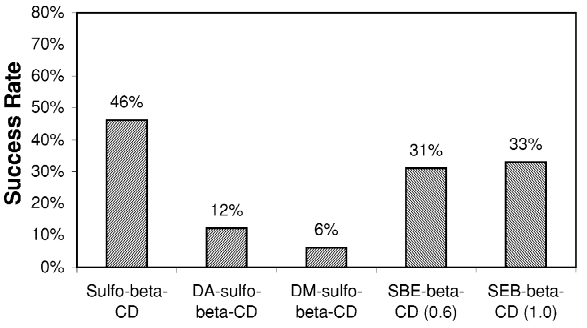

Fig. 6. Success rates of three single isomer sulfated β-CDs and two sulfobutyl-ether-β-CDs (SBE-β-CDs).

m*M*. It is not clear at this point whether the different concentration could result in a significant difference in the success rate between the HS-CDs from Aldrich and Beckman. A comparative study on the HS-CDs from Aldrich and Beckman is currently in progress.

Single isomer sulfated CDs are a series of isomerically pure sulfated CDs first developed by Vincent et al. at Texas A&M University [22]. The sulfate groups are attached to the CD molecules only at the C-6 hydroxyl position, and the C-2 and C-3 hydroxyl groups can also be modified with other functional groups, such as acetyl and methyl groups. Three single isomer sulfated CDs from Regis were tested in this study, namely, heptakis-6-sulfo-β-CD (Sulfo-β-CD), heptakis-(2,3-diacetyl-6-sulfo)-β-CD (DA-sulfo-β-CD), and heptakis-(2,3-dimethyl-6-sulfo)-β-CD (DM-sulfo-β-CD). Heptakis-6-sulfo-β-CD was the same sulfated CD derivative as HS-β-CDs from Aldrich and Beckman, except that the D.S. is much lower (1 vs. 1.7). The success rates of the single isomer sulfated-β-CDs are presented in Fig. 6. Compared to HS-β-CDs from Aldrich and Beckman, the success rate of heptakis-6-sulfato-β-CD (46%) was significantly lower, possibly due to its low D.S. In addition, the success rates for the other two single isomer sulfated-β-CDs, with the C-2 and C-3 hydroxyls modified with acetyl and methyl groups, were much lower than that of heptakis-6-sulfato-β-CD without modification at the C-2 and C-3 hydroxyl position. Obviously, the functional groups at the C-2 and C3 positions affects the selectivity of the single isomer sulfated CDs; however, more studies are needed to understand the effect of modification at the C-2 and C-3 position on the enantiomeric selectivity. It was also noticed that the majority of the model compounds did not elute within the migration window of 15 min. This may contribute to the low success rates of single isomer sulfated CDs.

Sulfobutyl-ether-β-CDs (SBE-β-CD) are essentially another group of sulfated CD derivatives [23]. The difference is that sulfate group is attached to the cyclodextrin rims through a butyl-ether linker in sulfobutyl-ether-β-CDs. Two sulfobutyl-ether-β-CDs with different degrees of substitution were tested against the model compounds, and their success rates are also shown in Fig. 6. The success rate of sulfobutyl-ether-β-CDs was much lower than that of highly sulfated CD derivatives as well as heptakis-6-sulfato-β-CD, but higher than that of the single isomer sulfated CD derivatives with diacetyl or dimethyl modification. In addition, the difference in the degree of substitution did not lead to any significant difference in the success rate of the SBE-β-CDs, and they showed similar selectivity for most of the model compounds.

Carboxymethyl-β-CD is another commonly used anionic CD derivative with a weakly ionizable carboxymethyl group attached to the cyclodextrin rim [24]. The degree of substitution of carboxymethyl-β-CD used in this study is not available from the supplier (Fluka). Carboxymethyl-β-CD was initially tested using reversed polarity, as was the case with all other anionic CDs. However, the model compounds could not be eluted within the separation window of 15 min. When the power

supply was switched to normal polarity, some model compounds started to elute with enantiomeric resolution. This might be attributed to weak ionization of carboxymethyl groups in the running buffer (pH~ 2.5). The success rate of carboxymethyl-β-CD was about 29%, similar to that of sulfobutyl-ether-β-CDs. It should be noted that carboxymethyl-β-CD acted essentially as a neutral CD derivative under the testing conditions (pH~2.5). Other experimental conditions (e.g., buffer pH) were not tested in this study, and the effectiveness of this CD derivatives could not be fairly judged based one condition. Its success rate might be significantly different at other pH values where it is fully ionized.

3.3. Results of confirmatory testing

The initial testing indicated that two highly sulfated CDs from Beckman (HS-β-CD and HS-α-CD) provided significantly higher success rates than other anionic CDs for the model compounds (68 and 62%). In addition, HP-β-CD (D.S.~0.95) from Aldrich yielded the highest success rate among the neutral CDs (42%). Even though the success rates of DM-β-CD and TM-β-CD from Fluka were very moderate (~30%), they could often provide complementary selectivity to HS-β-CD or HP-β-CD. With the five CD derivatives together (i.e., HS-α-CD, HS-β-CD, HP-β-CD, DM-β-CD, and TM-β-CD), over 90% of the 35 model compounds were resolved with a minimum resolution of 0.5 in the screening study. The initial experiment used only single injections in order to complete a large number of CE runs in a reasonable time period. To confirm the results of the initial testing, a confirmatory experiment was conducted on the identified CD derivatives using 20 different investigational drug compounds from J&J Pharmaceutical Research and Development.

The CE method and experimental conditions were the same as used in the initial testing, except that the run time was increased to 30 min. Table 2 lists the resolution for 20 test compounds and six commercial drug compounds using each of the four identified CD derivatives. The six commercial drug compounds were already tested in the initial screening, but included here as a measure of performance verification of the CE method. The resolution data for the six compounds as shown in Table 2 were similar to those obtained in the initial testing, indicating good reliability of the CE method.

Table 2
Resolution of test compounds using the four CD derivatives

Compound	HP-β-CD	DM-β-CD	HS-β-CD	HS-α-CD
RWJ-1	0.54	0	2.68	1.30
RWJ-2	np*	np	np	np
RWJ-3	np	np	np	0
RWJ-4	0.13	0	1.0	–
RWJ-5	0.61	0	>1.5	>1.5
RWJ-6	np	np	np	np
RWJ-7	np	np	np	np
RWJ-8	np	np	np	3.45
RWJ-9	–	–	0	0.54
RWJ-10	0	0.16	2.46	1.07
JRF-1	0	0	0.43	–
JRF-2	0.70	0	1.42	–
JRF-3	0	0	0.40	–
JRF-4	np	np	0	–
JRF-5	5.08	1.73	0	–
JRF-6	0	0	1.25	–
JRF-7	0.10	0.36	7.15	–
JRF-8	0.61	0.31	2.27	–
JRF-9	0.74	0.93	3.90	–
JRF-10	0.27	0	7.84	–
Terbutaline	3.09	0.84	3.04	0
Ofloxacin	1.51	2.06	11.5	0
Bupropion	1.20	0	3.77	9.78
Epinephrine	1.00	1.36	2.45	–
Miconazole	1.02	0	2.70	–
Tramadol	0	0	3.36	–

*np: No peak, indicating that the analyte did not elute within 30 min.

For 10 investigational drug compounds denoted as RWJ-1 to RWJ-10, five of them were resolved by HS-β-CD and HS-α-CD (Beckman) with the minimum resolution of 0.5. HP-β-CD (D.S.~1, Aldrich) resolved only two test compounds with the minimum resolution of 0.5; however, DM-β-CD (Fluka) was not able to resolve any of the test compounds. For 10 investigational drug compounds denoted as (JRF-1 to JRF-10), six of them were successfully resolved by HS-β-CD (Beckman) with a minimum resolution of 1.2, four test compounds by HP-β-CD (D.S.~1, Aldrich), and only two compounds by DM-β-CD (Fluka) at the minimum resolution of 0.5. Some experimental difficulties were encountered when testing these compounds with HS-α-CD (Beckman); therefore, the data for HS-α-CD on this set of test

compounds was not complete. The overall success rates for all 20 investigational drug compounds were 50% for HS-β-CD, 50% (based on 10 RWJ compounds tested) for HS-α-CD (Beckman), 32% for HP-β-CD (D.S.~1, Aldrich) and 11% for DM-β-CD (Fluka). The success rates for the four CD derivatives were lower in the confirmatory testing than those in the initial testing. However, the four CD derivatives together were still able to resolve 65% of the test compounds (13 out of 20). It was noticed that one JRF compound (JRF-4) and three RWJ compounds (RWJ-2, RWJ-3 and RWJ-6) were not resolved by any of the four CD derivatives. Compound JRF-4 was itraconazole and had very low solubility in the sample solvent. Compounds RWJ-2, RWJ-3 and RWJ-6 were structurally similar in that their chiral centers were at the same location in the molecular backbone. The inability of the four CD derivatives to resolve the three RWJ compounds contributed to the low overall success rate.

4. Conclusions

Five CD derivatives, i.e., HS-α-CD and HS-β-CD (Beckman), HP-β-CD (D.S.~1, Aldrich), DM-β-CD (Fluka) and TM-β-CD (Fluka) have been identified to have a wide range of enantiomeric selectivity towards basic pharmaceutical compounds in the current study. The five CD derivatives together were able to resolve over 90% of the model compounds in the initial testing and 60% of the test compounds in the confirmatory testing with a minimum resolution of 0.5 using a simple CE method. The CE method used in this study was by no means optimized for any test compounds, and the concentration of CD derivatives was set rather arbitrarily. It is realized that the change in the CD concentration could have significant effects on the enantiomeric separation of the test compounds. Nevertheless, the finding of this study can serve as general guidelines for method development. We recommend that HS-β-CD be used as the first choice for basic compounds in method development, the other identified CD derivatives (i.e., HS-α-CD, HP-β-CD, DM-β-CD and TM-β-CD) be also tested if HS-β-CD fails to provide desired resolution. The use of the five CD derivatives can reduce method development time tremendously by avoiding unnecessary testing of different chiral selectors.

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References

[1] J. Caldwell, J. Chromatogr. A 719 (1996) 3.
[2] M. Eichelbaum, A.S. Gross, Adv. Drug Res. 28 (1993) 1.
[3] G. Gubitz, M.G. Schmid, Biopharm. Drug Dispos. 22 (2001) 291.
[4] K.D. Altria, M.A. Kelly, B.J. Clark, Trends Anal. Chem. 17 (1998) 214.
[5] H. Nishi, Electrophoresis 20 (1999) 3237.
[6] G. Blaschke, B. Chakvetadze, J. Chromatogr. A 875 (2000) 3.
[7] A. Amini, Electrophoresis 22 (2001) 2107.
[8] C.J. Shaw, N.A. Guzman, in: L. Ohannesian, A.J. Streeter (Eds.), Handbook of Pharmaceutical Analysis, Marcel Dekker, New York, 2002, p. 313.
[9] M.M. Rogan, K.D. Altria, in: K.D. Altria (Ed.), Capillary Electrophoresis Guidebook: Principles, Operation, and Application, Humana Press, Totowa, NJ, 1996, p. 171.
[10] A. Guttman, in: J.P. Landers (Ed.), Handbook of Capillary Electrophoresis, 2nd ed., CRC Press, New York, 1997, p. 75.
[11] K. Verleysen, P. Sandra, Electrophoresis 19 (1998) 2798.
[12] S. Fanali, J. Chromatogr. A 875 (2000) 89.
[13] M. Fillet, P. Hubert, J. Crommen, J. Chromatogr. A 875 (2000) 123.
[14] T. De Boer, R.A. De Zeeuw, G.J. De Jong, K. Ensing, Electrophoresis 21 (2000) 3220.
[15] J. Mosiner, V. Tomankova, I. Nemcova, J. Zyka, Anal. Lett. 34 (2001) 1979.
[16] T. Loftsson, M.E. Brewster, J. Pharm. Sci. 85 (1996) 1017.
[17] M. Fillet, P. Hubert, J. Crommen, Electrophoresis 19 (1998) 2834.
[18] L. Zhou, R. Thompson, S. Song, D. Ellison, J.M. Wyvratt, J. Pharm. Biomed. Anal. 27 (2002) 541.
[19] B. Lin, X. Zhu, B. Kopenhoefer, U. Epperlein, LC·GC 15 (1997) 43.
[20] K.W. Phinney, J.W. Jackson, L.C. Sander, Electrophoresis 23 (2002) 1308.

[21] F.T.A. Chen, G. Shen, R.A. Evangelista, J. Chromatogr. A 924 (2001) 523.
[22] J.B. Vincent, A.D. Sololowski, T.V. Nguyen, G. Vigh, Anal. Chem. 69 (1997) 4226.
[23] R.J. Tait, D.O. Thompson, V.J. Stella, J.F. Stobaugh, Anal. Chem. 66 (1994) 4013.
[24] J. Zukowski, V. De Biasi, A. Berthod, J. Chromatogr. A 948 (2002) 331.