Optimization of a capillary electrophoresis method to determine the chiral purity of a drug

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Abstract

Capillary electrophoresis (CE) using hydroxypropyl-β-cyclodextrin (HP-β-CD) in the separation buffer was investigated to determine the overall chiral purity of a drug containing a single stereogenic center. The effects of primary factors —pH, buffer components, buffer concentration, cyclodextrin concentration and sample amount (concentration and injection volume)— on the resolution of the enantiomers were investigated. Secondary factors such as the HP-β-CD source, lot and degree of substitution that were expected to affect the robustness of the assay were investigated also. The linearity, quantitation limit for the trace enantiomer and the precision of the measurements were determined. This study shows that understanding and optimizing the assay conditions leads to a chiral CE separation that is comparable to that obtained by chiral HPLC. However, chiral CE separations achieved with buffer additives have the advantages of shorter run times, higher numbers of theoretical plates (greater resolution), smaller amounts of chiral additive (less cost) and greater ruggedness (separation virtually independent of column properties unlike HPLC).

1. Introduction

The introduction of new drugs requires development of assays to control identity, potency or strength, and purity. In addition, their stability is followed with time to observe the formation of degradation products and the concomitant loss in potency and purity. For drugs that contain one or more stereogenic centers, the chiral purity may be an important component in the determination of purity and stability.

A variety of chiral assay techniques are available to the analyst [1]. These include spectroscopic techniques (optical rotation, NMR, IR and X-ray diffraction), chromatographic methods (GC, HPLC and TLC) and physical properties

(melting range). For instance, chiral HPLC is a very popular and powerful tool that can be used for the control of bulk drug substances (active ingredient) and drug products (formulated drug). The choice of chiral HPLC methods is based on their ability to give baseline resolution of the trace enantiomer from the major enantiomer and to detect and quantify the trace enantiomer at relatively low levels (approximately 0.1 to 1.0%). However, it is difficult to develop chiral HPLC methods that are robust (or rugged). The lack of robustness arises from changes in the properties of columns with time, poor column-to-column reproducibility and the relatively low efficiency obtained from most chiral columns. In addition, it may be difficult to find HPLC separation conditions where the trace enantiomer elutes before the main component —a condition

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that more readily allows maximum sensitivity for a component that has significant tailing [2]. Finally, it would be desirable to determine both non-chiral and chiral purity in one separation. It has been very difficult to accomplish this task with chiral HPLC columns because they typically have low chemical selectivity and efficiency [1], although the use of chiral mobile phase additives may be an alternative.

Capillary electrophoresis (CE) can achieve chiral separations [3–7]. Buffer additives were used in most of these previous investigations. The use of buffer additives gives maximum flexibility to control the type and amount of chiral selector. Cyclodextrins are one of the most useful chiral selectors; they are available in a variety of cavity sizes and substitutions. The cavity size and geometry determine whether an inclusion complex can be formed whereas the substituent and substitution pattern affect the strength of the complex by changing the hydrophobicity of the cyclodextrin and its ability to form hydrogen bonds. Many groups have employed neutral cyclodextrins, but some have used charged cyclodextrins, chiral crown ethers or other chiral resolving agents [3,5,8–10]. In other cases, separations were achieved by micellar electrokinetic capillary chromatography (MECC) using bile salts, other chiral surfactants, or cyclodextrins combined with achiral surfactants [3–7].

Other investigators have looked specifically at quantitative aspects [11] and performed an inter-laboratory cross-validation of a chiral CE method [12]. To be useful for the routine analysis of pharmaceuticals in a control laboratory, CE must demonstrate attributes that are similar to or better than those obtained from chiral HPLC separations. For example, CE potentially offers faster separations, higher resolution (more theoretical plates) and much less dependence on column properties (column surface reproducibility). In addition, its utility depends upon the ability to rapidly develop optimized separations.

The physical properties that describe a chiral CE separation using cyclodextrin can be summarized as (1) the enantiomers have the same electrophoretic mobility, (2) the enantiomers and the chiral selector are in rapid equilibrium

with the enantiomer–chiral selector complex, (3) the two enantiomers have slightly different complex formation constants and (4) the enantiomer–chiral selector complexes have very similar electrophoretic mobilities that are different (smaller for neutral cyclodextrins) from the free enantiomers. Under these conditions, the separation that will be achieved is determined by the proportion of time that the enantiomers exist free (uncomplexed) in solution compared to the proportion of time that they exist as the complex. That is, specificity (or selectivity) is determined almost completely by the difference in formation constants. However, the maximum attainable resolution is a function of both the specificity and the peak width. Therefore, this work reports peak separation as a measure of selectivity, tailing as a measure of peak width, and resolution.

Wren and Rowe [13–16] and Rawjee and coworkers [17,18] have presented models for the formation of enantiomeric complexes and their subsequent separation by CE. In a number of papers [19–24] various experimental factors were optimized. However, the importance of these factors is not broadly recognized in the scientific community. This work was performed to further decipher the effect of experimental variables on the resolution as well as to determine analytical validation parameters —such as linearity, precision and sensitivity— for separations that use neutral cyclodextrins.

A screen of numerous neutral and charged cyclodextrins [25] identified hydroxypropyl-β-cyclodextrin (HP-β-CD) as the preferred derivative for the separation of the enantiomers of LY248686 (Fig. 1), a secondary amine. This study investigated the effect of separation buffer pH, buffer composition, buffer concentration and HP-β-CD concentration on the separation. It also addresses the issues of precision, sensitivity, and robustness.

2. Experimental

LY248686 (S enantiomer) as the hydrochloride salt and the racemate of LY248686 as the maleate salt were obtained from Eli Lilly (Lilly
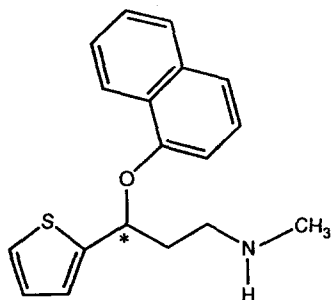
Fig. 1. Structure of LY248686 (R enantiomer), (+)-N-methyl-γ-(1-naphthalenyloxy)-2-thiophenepropanamine hydrochloride.

Research Labs., Indianapolis, IN, USA). HP-β-CD used in the optimization was obtained from Astec (Whippany, NJ, USA). Additional samples of HP-β-CD were obtained from Pharmos (Alachua, FL, USA), Aldrich (Milwaukee, WI, USA), American Maize-Products (Hammond, IN, USA), and ICN Biochemicals (Cleveland, OH, USA). Hydroxypropylmethyl cellulose and hexadecyltrimethyl-ammonium bromide were obtained from Sigma. Purified water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA). All other chemicals were analytical grade or equivalent.

The data were obtained on an Applied Biosystems (Santa Clara, CA, USA) Model 270HT or a Beckman Instruments (Palo Alto, CA, USA) P/ACE System 2100 capillary electrophoresis instrument as indicated. Capillaries were obtained from Polymicro Technologies (Model 270HT; Phoenix, AZ, USA) or Beckman (System 2100). Separation conditions are indicated and UV detection at 214 nm was employed. Analog data were collected directly from the absorbance detector on an in-house chromatography computer system that has storage, manipulation and graphics capabilities. Resolution and tailing were estimated using the USP [26] and Foley and Dorsey [27] definitions, respectively; they are given by

$$\text{USP resolution} = 2(t_2 - t_1)/(W_1 + W_2) \text{ at baseline} \tag{1}$$

$$\text{Dorsey tailing} = W/[2(F - t)] \text{ at 10\% height} \tag{2}$$

where t_1 and t_2 are the migration times of peaks 1 and 2, W is the peak width measured by extrapolation of the peak tangent to the baseline or at 10% of the peak height as indicated, and $(F - t)$ is the time between when the signal reaches 10% of its value at the peak maximum (on the front edge of the peak) and the time of the peak maximum (migration time).

3. Results and Discussion

Enantiomers can be separated by formation of static or dynamic diastereomers that have different physiochemical properties. Even when the diastereomers have nearly the same properties, one can obtain specificity by CE when there is a differential formation of a dynamic diastereomer complex. Cyclodextrin complexes formed from two enantiomers will have the same charge, essentially the same size, and, therefore, essentially the same electrophoretic mobility. However, it is possible to separate the enantiomers when the formation constants of the diastereomeric complexes are sufficiently different and when conditions are chosen such that the average time that the drug spends as a complex is different for the two enantiomers. That is, the average mobility of the drug will be determined by the mobility of the free drug, the mobility of the complex and the proportion of time that the drug spends in each form. If that proportion differs for the two enantiomers, then their average mobility will be different and separation can be achieved even though the mobilities of the diastereomer complexes are essentially identical.

When neutral cyclodextrins are used, the compound must be charged for the complex to have a non-zero electrophoretic mobility. At the same time, the elution of neutral impurities from the column requires that the pH be high enough to provide a reasonable electroosmotic flow. The former is required to determine chiral purity and the latter to determine achiral purity. For LY248686, this means that the pH of the separation buffer must be at least moderately less than the pK_a of the drug to produce the cationic species but sufficiently high to give a reasonable electroosmotic flow. The pK_a of LY248686 in

dimethylformamide–water (66:34) is 9.6; the corresponding aqueous pK_a value is above 8.5 but cannot be determined accurately due to the limited aqueous solubility at high pH. The chiral separation was acceptable at pH values less than about 4.5 (Fig. 2). The decrease in resolution above pH 4.5 is probably due to the higher electroosmotic flow velocity that gives insufficient separation time. However, even at pH 4.5, the electroosmotic flow was insufficient to elute the neutral complexes that would be formed if neutral impurities were present [25]. Because we could not get elution of the neutral species at any pH where the enantiomers were separated, the remainder of the work was performed at pH 2.35 where the resolution of the enantiomers was best.

Various buffer components were investigated to determine their effect on the separation. At pH 2.35, phosphate and Tris–phosphate buffers gave similar separations and were superior to separations obtained with glycine. Additionally, a variety of silica surface modifiers were examined. Various groups have examined the effect of surfactants or organic solvents [3,22,28,29] that reduce the electroosmotic flow and, therefore, provide a longer time to discriminate be-

tween the diastereomeric complexes and give a better separation. In this study, the addition of cellulose derivatives such as hydroxypropyl-methyl cellulose (HPMC-50 or HPMC-4000) or large, non-polar substances such as hexade-cyltriammonium bromide (HTAB) did not enhance the separation of the enantiomers.

Although the effects of buffer concentration and ionic strength have been studied for non-chiral CE separation applications [30], only a little information has been reported about their effects in chiral separations [19,23,24,31,32]. Increasing buffer strength or ionic strength will modify the hydrophobic interactions of the enantiomers with the cyclodextrin. However, it is not clear how this change will, by itself, improve the selectivity or efficiency of the separation. On the other hand, increasing the buffer concentration reduces the electroosmotic flow because it reduces its driving force, the zeta potential. Longer migration times, in turn, provide a longer time for enantiomer-chiral selector discrimination. That is, longer times within the electric field where the two complexes have slightly different mobilities will give a greater absolute separation of the two species. Additional band broadening during the longer separation
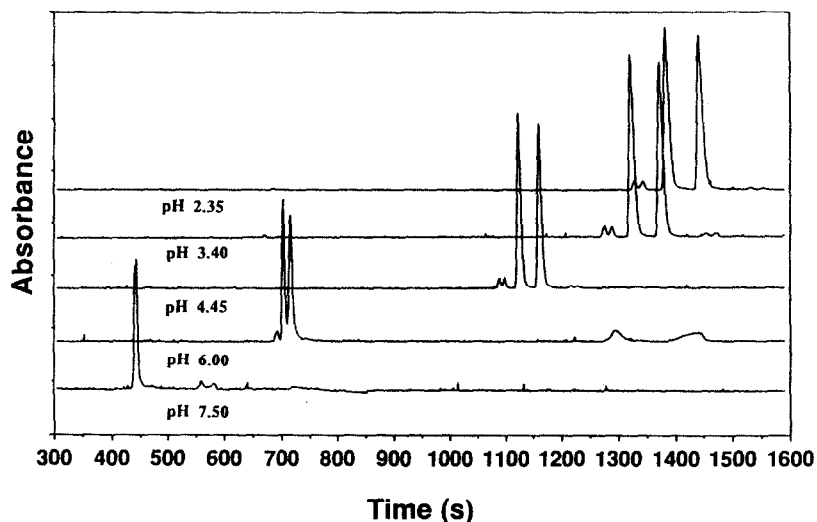

Fig. 2. Separation of the racemic mixture of LY248686 using 100 mM Tris–phosphate buffers at the indicated pH. Other separation conditions were about 4 mM HP-β-CD using a ABI 270HT instrument with a 75 cm (50 cm to the window) × 50 μm I.D. silica capillary, 23 kV, 30°C and 214 nm detection.

will be relatively small so that the principal disadvantage of reduced electroosmotic flow is an increase in analysis time. Greater resolution and longer separation times with increasing buffer concentration are apparent in Fig. 3; the resolution, tailing and peak separation are quantitated in Table 1. The maximum usable buffer concentration is limited by the conductivity of the buffer. When the current is large enough that it produces more Joule heat than can be dissipated, the separation efficiency is compromised. Thus, it is advantageous to use low-conductivity buffers, such as zwitterionic buffers, and small-diameter capillaries when possible.

The concentration of the chiral selector is important. At the low concentration extreme, there is not enough chiral selector available to form complexes and, therefore, no separation of enantiomers is possible. At the high concentration extreme (experimentally limited by solubility of the chiral selector), both enantiomers are completely complexed. Because both enantiomer–chiral selector complexes have very similar mobilities, no separation of enantiomers is possible in this case either. Only in the midrange of concentrations will an adequate separation occur. Wren and Rowe have thoroughly

Table 1
Effect of Tris–phosphate buffer concentration on resolution and peak separation

Buffer concentration (mM)	Resolution	Tailing	Peak separation (s)
10	2.17	2.24	36
20	2.80	2.09	48
50	3.69	1.72	61
100	4.02	1.86	73
200	4.18	1.61	83

Conditions: ABI 270HT instrument; 75 cm (50 cm to the window) × 50 μm I.D. silica capillary; 23 kV (307 V/cm); 1-s vacuum injection (3.9 nl) of a 0.107 mg/ml sample solution; Tris–phosphate pH 2.35 separation buffer at the indicated concentration that contained about 4.2 mM of HP-β-CD. Tailing given for the first peak.

discussed the interaction of one species with a chiral selector. Their work gives predictions of what concentration will give the best peak separation [13–15] or resolution [16]. The chiral selector concentration that gives the maximum separation depends upon the specific physical interaction, that is, the strength of the enantiomer–chiral selector complex. The concentration that gives the maximum resolution is predicted to be slightly different. A non-symmetrical curve
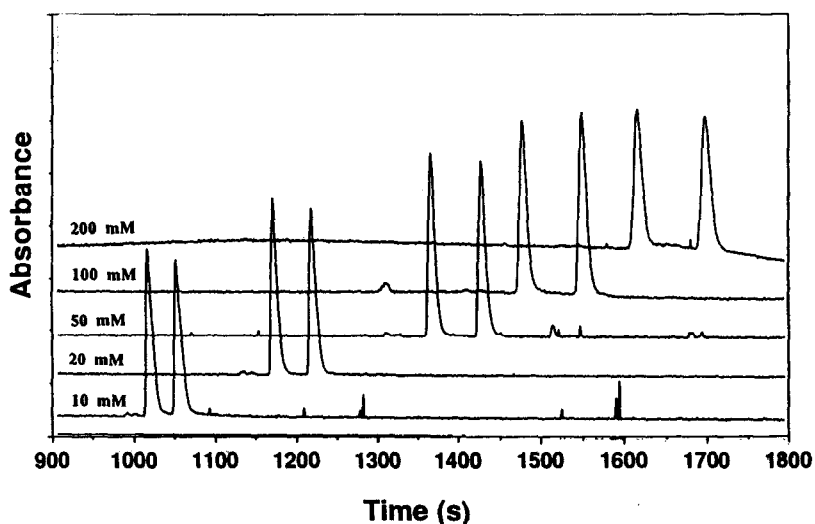

Fig. 3. Separation of the racemic mixture of LY248686 at various concentrations of Tris–phosphate buffer at pH 2.35. Other conditions as in Fig. 2.

that has a steep rise from low concentrations and a longer tail to high concentrations is predicted for peak separation or resolution versus chiral selector concentration. The maximum peak separation is determined by the ratio of the two complex formation constants; it occurs when the chiral selector concentration is equal to the reciprocal of the average complex formation constant. A more extensive description of the theory for resolution that allows both the neutral and charged species to interact with the chiral selector is given by Rawjee and co-workers [17,18]. However, this complexity is not needed to explain the results obtained in this study.

The behavior exhibited by LY248686 (Fig. 4) and the resolution (Table 2) agree with the predictions of Wren and Rowe. The observed peak shape, as measured by peak tailing (Table 2), also varies somewhat with cyclodextrin concentration. Peak shape has not been discussed in the theoretical treatments for chiral separations. One could rationalize that peaks would be sharpest at the extremes of cyclodextrin concentrations when the analyte is either free or completely complexed, but there may be other explanations.

The observations discussed above are directly

Table 2
Effect of HP-β-CD concentration on resolution and peak separation

HP-β-CD concentration (mM)	Resolution	Tailing	Peak separation (s)
1.07	2.31	1.9	38
2.01	2.82	2.0	50
4.39	3.05	2.3	58
6.47	2.55	2.2	54
9.78	2.16	1.8	46

Conditions: ABI 270HT instrument; 72 cm (50 cm to the window) × 50 μm I.D. silica capillary; 22 kV (306 V/cm); 2-s vacuum injection (8.1 nl) of a 0.107 mg/ml sample solution; 50 mM Tris–phosphate pH 2.35 separation buffer that contained the indicated concentration of HP-β-CD. Tailing given for the first peak.

related to the concentration of the cyclodextrin in the separation buffer. However, the dynamic equilibrium is affected by the molar ratio of the complexing agent (chiral selector) to the analyte (enantiomer) within the actual sample zone. For a given cyclodextrin concentration, this ratio is governed by the sample concentration and the volume of sample solution injected. There are no previous reports of the effects of sample con-
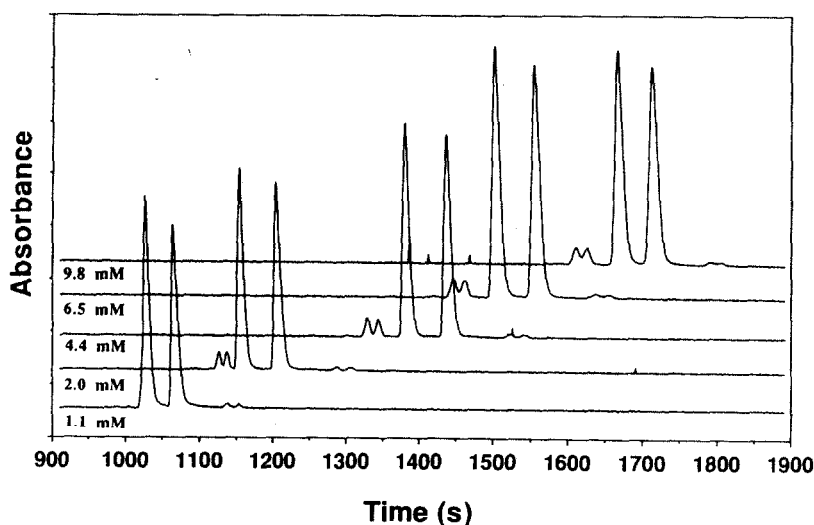

Fig. 4. Separation of the racemic mixture of LY248686 at various concentrations of hydroxypropyl-β-cyclodextrin in 50 mM pH 2.35 Tris–phosphate buffer. Other conditions as in Fig. 2.

Table 3
Effect of sample concentration on resolution and peak separation

Sample concentration (mg/ml)	Experiment 1			Experiment 2		
	Resolution	Tailing	Peak separation (s)	Resolution	Tailing	Peak separation (s)
0.001	3.80	0.9	49	–	–	–
0.002	4.66	1.6	46	–	–	–
0.005	4.10	0.8	46	–	–	–
0.010	3.70	1.1	45	6.53	1.1	113
0.020	4.32	1.2	45	4.82	1.1	117
0.050	3.77	1.3	44	5.10	1.4	118
0.100	3.45	1.7	44	4.53	1.7	120
0.200	2.88	2.8	44	3.54	2.5	122
0.50	2.20	5.4	46	2.53	4.9	126
1.00	1.60	–	46	2.00	17.2	129

Experiment 1: ABI 270HT instrument; 75 cm (50 cm to the window) × 50 μm I.D. silica capillary; 23 kV (303 V/cm); 1-s vacuum injection (3.9 nl) of the indicated sample solution; 4.00 mM HP-β-CD in 50 mM Tris–phosphate pH 2.35 separation buffer. Tailing given for the first peak. *Experiment 2*: Beckman P/ACE System 2100 instrument; 57 cm (50 cm to the window) × 50 μm I.D. silica capillary; 17.4 kV (305 V/cm); 5-s pressure injection (3.9 nl) of the indicated sample solution; 4.00 mM HP-β-CD in 100 mM Tris–phosphate pH 2.35 separation buffer. Tailing given for the first peak.

centration or volume on chiral resolution. There-fore, the resolution, tailing and peak separation were examined as a function of the sample concentration and volume injected.

The data indicated that the resolution was essentially constant at low sample concentrations but decreased extensively at high sample concentrations (Table 3). (The decrease for the lowest sample concentration is most likely due to the imprecision in measuring the resolution for such small peaks.) The change in resolution with sample concentration was even greater than its change with the cyclodextrin concentration (Table 2) at least partly because the sample concentration was changed over a greater range (1000× range in one experiment and 100× in the second) than was the HP-β-CD concentration (9× range). In contrast to the behavior observed with changes in cyclodextrin concentration, the peak separation remained nearly constant but the peaks became much more asymmetrical when the sample concentration was varied (ABI instrument). The second instrument (Beckman) showed similar effects for resolution and asymmetry, but peak separation showed a great-er change. Large sample volumes produced a

decrease in resolution but no change in peak separation on the Beckman instrument (Table 4). Again, peak asymmetry as measured by tailing increased extensively at large sample volumes. Injection of the largest sample (3.7% of the total column volume) would be expected to increase the peak width but not necessarily to cause the asymmetrical peaks. Changes in res-

Table 4
Effect of sample volume on resolution and peak separation

Volume injected (nl)	Resolution	Tailing	Peak separation (s)
1.04	4.54	1.3	94
2.08	4.55	1.4	93
5.21	4.32	1.8	95
10.4	3.48	2.4	96
20.8	2.57	3.6	94
41.7	2.00	7.8	95

Conditions: Beckman P/ACE System 2100 instrument; 57 cm (50 cm to the window) × 50 μm I.D. silica capillary; 17.4 kV (305 V/cm); 1, 2, 5, 10, 20 and 40-s pressure injections of a 0.100 mg/ml solution to give the indicated volumes; 4.00 mM HP-β-CD in 100 mM Tris–phosphate pH 2.35 separation buffer. Tailing given for the first peak.

olution and asymmetry were apparent with in-
jections as small as 0.47% of the column volume
(5.2 nl).

The molar ratio of cyclodextrin to analyte for
the analyte zones can be calculated by measuring
the peak width and assuming an average con-
centration within the peak. These calculations
suggest about a 15:1 molar ratio of cyclodextrin
to analyte for typical conditions with extremes of
about 2600:1 for the very dilute analyte con-
centrations to about 3:1 or 4:1 for very low
cyclodextrin concentrations or high sample con-
centrations. The overall conclusion is that the
molar ratio, analyte concentration and sample
volume interact to give differences in resolution,
peak shape and peak separation. There may be
some instrument-dependent behavior as well.
However, there clearly are optimum combina-
tions of cyclodextrin concentration combined
with moderate sample concentrations and in-
jection volumes that give the best separations.

These results demonstrate that choices for the
chiral selector and its concentration, pH and
concentration of separation buffer, sample con-
centration and injection volume affect the res-
olution. However, other factors will contribute

to its utility within a control laboratory environ-
ment. For example, the lot-to-lot or manufac-
turer-to-manufacturer variability of the cyclodex-
trin or the degree of substitution can affect its
ability to complex with the enantiomers and,
therefore, the robustness of the assay. A pre-
liminary investigation of these factors was under-
taken also.

Various sources of HP-β-CD are compared in
Fig. 5. In all cases, the R enantiomer (first peak)
was separated from the S enantiomer. In some
cases, however, one of the enantiomers of an
impurity was not completely separated from the
trace R enantiomer of the drug, and therefore it
would be difficult to determine the chiral purity
of the drug. Further investigations showed only
very minor variation in the lot-to-lot consistency
from one supplier (Fig. 6) but definite variation
with the degree of substitution (DS) (Fig. 7).
The variation in the average degree of substitu-
tion of derivatized cyclodextrins and the multiple
components affect CE separations [22,33,34].
Clearly, variation due to supplier, lot-to-lot
consistency and/or degree of substitution need
further investigation.
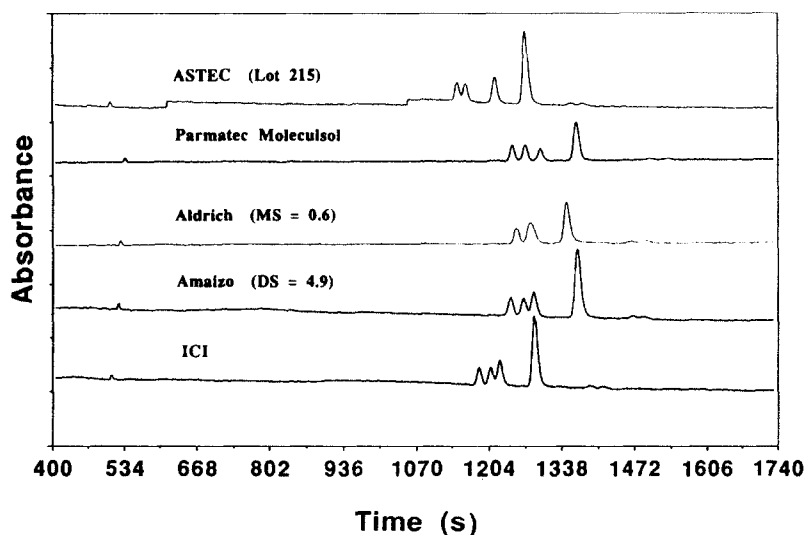
The sensitivity of CE for the detection of

Fig. 5. Effect of HP-β-CD source on the separation of the R and S enantiomers of LY248686 and a potential impurity.
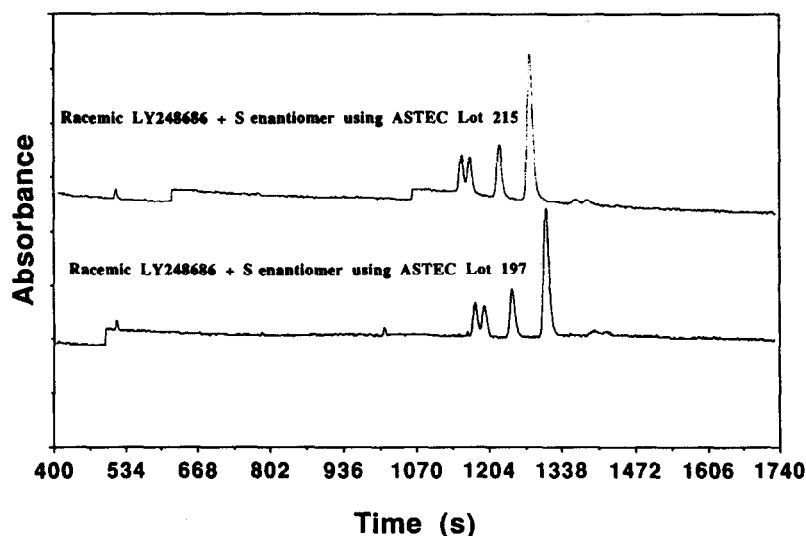Separation conditions as in Table 4 with a 5-s pressure injection.

Fig. 6. Lot-to-lot consistency of HP-β-CD on the separation of the R and S enantiomers of LY248686 and a potential impurity. Separation conditions as in Fig. 5.

impurities is limited primarily by the dynamic range of the experiment. Unlike RP-HPLC, CE columns cannot be overloaded to maximize sensitivity without suffering severe losses in resolution. However, others have reported quantitation limits for chiral separations of about 1% with detection limits down to about 0.3% [35,36]. Non-chiral CE methods have achieved sensitivities about an order of magnitude lower [37–39]. A standard addition experiment (Fig. 8) indicated a quantitation limit for the R enantiomer to be about 0.1–0.2%, about the same as that obtained from a chiral HPLC separation

(data not shown). Furthermore, extrapolation of the standard addition plot indicated an initial content of R enantiomer of about 0.4% using either peak area ratios (corrected for differences in electroosmotic velocities) or by comparison to an external standard. This number is only slightly higher than that estimated from the chiral HPLC separation (about 0.2%). Linearity of the standard addition experiment was excellent (coefficient of determination was 0.995).

The precision of the measurements is given in Table 5. As expected, much better precision for peak times and peak areas is obtained by using

Table 5
Precision of chiral CE experiments for racemic LY248686

Instrument	Replicates	S Enantiomer migration time (s)	S Enantiomer peak area	Migration time ratio	S Enantiomer (%)
ABI	9	1188 (0.40%)	173.2 (1.31%)	0.960 (0.03%)	48.4% (0.30%)
Beckman	27	1417 (1.85%)	1633 (6.0%)	0.961 (0.12%)	49.1% (1.6%)

Precision data with R.S.D. indicated in parentheses. ABI instrument; conditions as in Table 3 with 0.107 mg/ml sample concentration. The S enantiomer (%) refers to the S enantiomer content of the "racemic" mixture determined by CE and the indicated precision (R.S.D., %). Beckman instrument: conditions as in Table 3 with 0.12 mg/ml sample concentration.
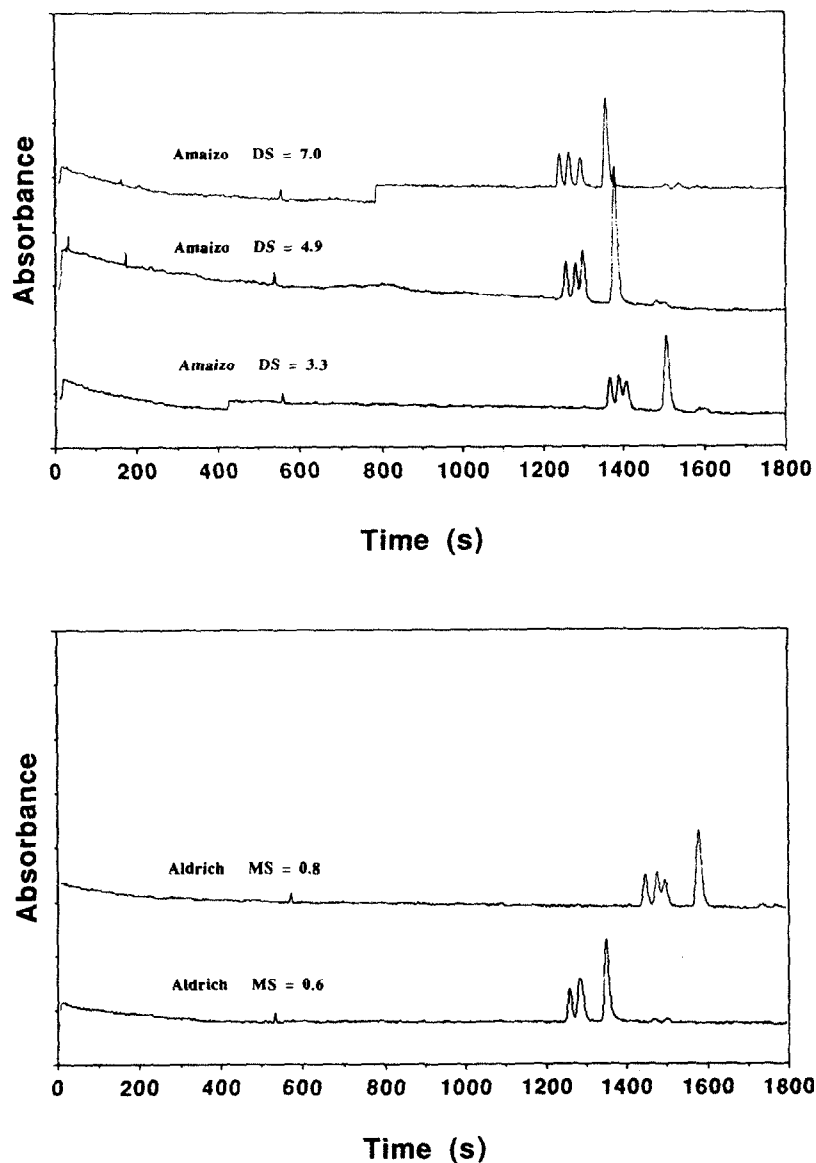
Fig. 7. Effect of degree of substitution of HP-β-CD on the separation of the R and S enantiomers of LY248686 and a potential impurity. Separation conditions as in Fig. 5.

ratios rather than absolute values. The precision observed for the ABI instrument for both migration times and peak areas is comparable to that reported by others [34,38] including an inter-laboratory study [12]. In addition, the precision using ratios from the ABI instrument is compar-

able to that obtained by typical HPLC experiments. The results obtained on the Beckman instrument were poorer than expected although it should be noted that there were some differences in conditions, e.g., the capillary dimensions and applied voltages were different and the
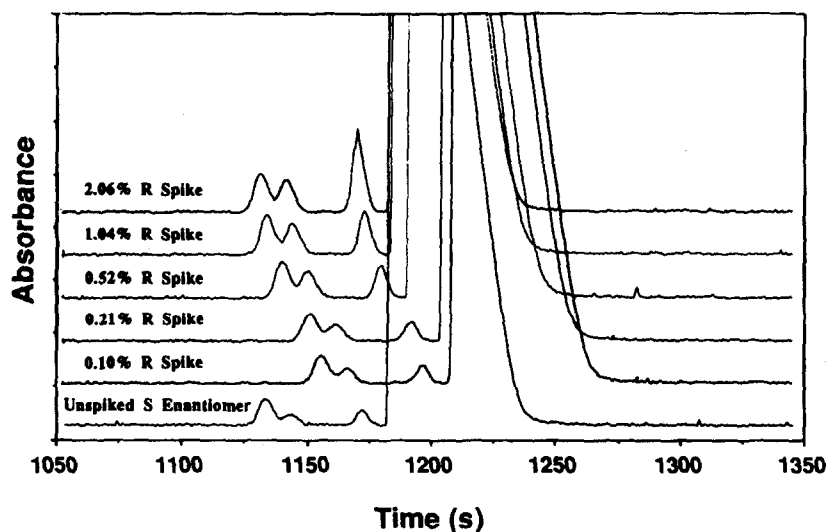
Fig. 8. Standard addition of S enantiomer to LY248686. Separation conditions as in Table 3 using the ABI 270HT instrument.

migration times were longer. Also, the greater number of replicates made it more sensitive to time dependent drift. Certainly others have reported similar results for both instruments.

The selectivity obtained with the optimum chiral separation conditions is illustrated in Fig. 9. All the analogues and potential degradation products that were available are well resolved from the enantiomers of interest except for the dimethyl analog that migrates between the two enantiomers. If it were present in significant amounts, it would limit the usefulness of the method. However, this potential impurity and neutral analogues do not comigrate with the
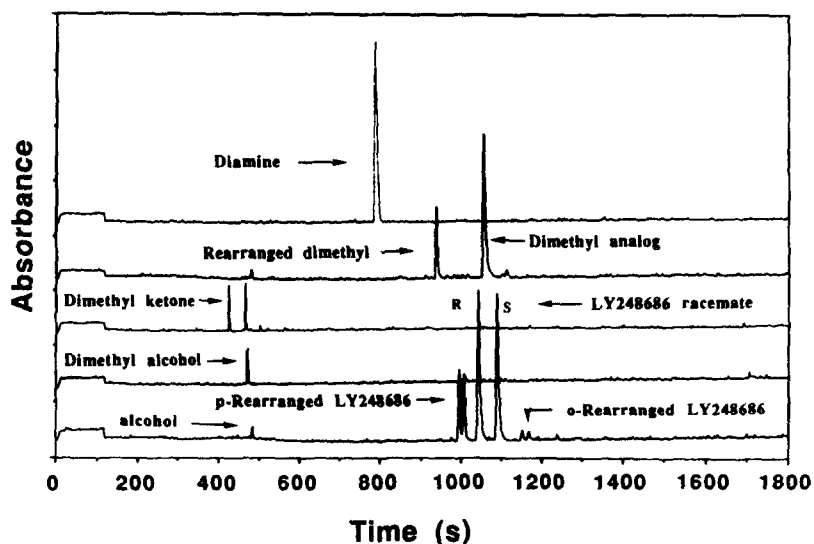

Fig. 9. Chiral separation of analogues and potential degradation products from LY248686. Separation conditions as in Table 3 (Beckman instrument) except 25 kV separation potential.

trace enantiomer (the isomer that is to be controlled) and, thus, do not prevent its quantitation.

4. Conclusions

Our investigation indicates that the first step in the development of an enantiomeric separation with a neutral cyclodextrin is to select an appropriate buffer to give a pH where the analyte will be charged. The second step involves evaluating and selecting a chiral selector that readily forms a complex with the charged drug. Although HP-β-CD was found to be best to separate LY248686 from its enantiomer, it will not always be the best selector.

Then, the concentrations of the buffer and cyclodextrin can be optimized to achieve maximum separation or resolution. Generally, high buffer concentrations reduce the electroosmotic flow and tend to give better separations at a cost of increased analysis time and higher currents. Currents can be minimized by the choice of low conductivity buffers, such as Tris–phosphate or zwitterionic species rather than purely ionic components such as phosphate. Cyclodextrin concentration must be adjusted to give an optimal differential partitioning of the two enantiomers between their free form and their complexed form. Partitioning and, thus, separation are affected not only by the bulk cyclodextrin concentration but also by the quantity of sample injected (concentration and volume). High sample concentration and high injection volume adversely affect peak shape and resolution. Cyclodextrin source, lot and degree of substitution are important secondary contributors to the resolution and to the robustness of the assay. These latter variables need further exploration. With the knowledge gained from these experiments, an experimental design approach (e.g., Plackett–Burman) could be used to better determine the interactions of all the factors on separation. Finally, it was demonstrated that CE has adequate sensitivity and reproducibility to monitor the chiral purity of a drug.

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