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Systematic screening approach for chiral separations of basic compounds by capillary electrophoresis with modified cyclodextrins

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Abstract

A simple, systematic method was developed for rapidly screening potential capillary electrophoresis (CE) separation conditions for small, amine-containing enantiomers. During method development, 39 pairs of enantiomers were investigated and partial or complete separation was achieved in every case. Baseline resolution was achieved by these initial screening conditions in over half of the cases. The screening strategy uses a bare fused silica capillary and a pH 2.5 amine-modified phosphate buffer containing one of the selected cyclodextrins (CD): dimethyl-β-CD, hydroxypropyl-β-CD, hydroxypropyl-α-CD, hydroxypropyl-γ-CD and sulfated-β-CD. An additional set of compounds have been screened by this approach to demonstrate the validity of the method. The paper outlines the experimental work carried out to develop the screen and describes how one might implement it for a new compound.

Keywords: Capillary electrophoresis; Chiral separation; Enantiomers; Screening; Cyclodextrins

1. Introduction

The application of capillary electrophoresis (CE) to chiral separations has progressed dramatically in recent years. This progress is due to several factors, including the increased availability and ruggedness of commercial CE instruments, increased importance of chiral purity in pharmaceutical development, and some general advantages of CE over HPLC (e.g. cost, efficiency, and widely variable separation conditions). As a result, guidelines for rapid development of chiral separation conditions by CE are becoming more widely sought, and there have been several reports offering some such guidance [1–14], both theoretical and empirical. Theoretical approaches are useful in providing general directions (e.g. whether to start with a low or high pH, or whether a crown ether is a potential chiral selector). To date, however, no universal model exits which allows one to accurately predict appropriate separation conditions a priori, given simply the structure of the analyte. Thus, empirical screening guidelines remain a very valuable and practical tool.

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Cyclodextrins are the most widely used chiral selectors in CE [1,2,15–17]. They have negligible UV absorbance, are water-soluble, and are readily available in a large number of modified forms. The enantioselective recognition results from inclusion of a hydrophobic portion of the solute in the CD cavity and also from hydrogen bonding to the chiral hydroxyl moieties.

The objective of this work was to develop a fast, simple, and effective CE screening method for the chiral separation of small, basic compounds, and evaluate it on both commercial and Lilly compounds. The goal was to start from the currently available literature and devise a screening approach which would use inexpensive bare silica capillaries, simple cyclodextrin-containing run buffers, and which would be straightforward and highly effective. Also, the intent was to use a large number of analytes for the development and evaluation of our screening approach. Although screening approaches have been reported elsewhere, most either use specialized capillaries (e.g. neutral-coated), a variety of pH conditions, or have not been evaluated on a large number of compounds. Furthermore, a greater variety of cyclodextrins have become available in recent years which are potentially useful for screening purposes; particularly, sulfated cyclodextrins and modified alpha- or gamma-cyclodextrins.

The results obtained with this new screening methodology compared favorably to those obtained with neutral capillaries. The data are also similar to those reported under optimal conditions although no attempt was made here to optimize the separations. Among 49 pairs of enantiomers studied, 47 pairs achieved partial or complete separations, and 27 pairs had resolutions ≥ 1.4, without further optimization.

2. Experimental

2.1. Chemicals

Hydroxypropyl-β-cyclodextrin (HP-β-CD, D.S. = 5.6), phosphoric acid, and tetrabutylammonium hydroxide (TBA) were obtained from Aldrich (Milwaukee, WI). Hydroxypropyl-α-CD (HP-α-CD, D.S. = 3.3), hydroxypropyl-γ-CD (HP-γ-CD, D.S. = 4.6) and sulfated-β-CD (Sul-β-CD, D.S. = 4.0) were obtained from Cerestar (Hammond, IN). Triethylamine (TEA) was obtained from Fisher (Pittsburgh, PA). Dimethyl-β-CD (DM-β-CD) and all commercially available chiral compounds were obtained from Sigma (St. Louis, MO). The rest of the chiral compounds were internal to Lilly Research Laboratories.

2.2. Buffer and sample preparation

A phosphate buffer for neutral cyclodextrins was prepared by adjusting the pH of 30 mM phosphoric acid to 2.5 with TBA. A phosphate buffer for sulfated-β-CD was prepared by adjusting the pH of 25 mM phosphoric acid to 2.5 with TEA. Run buffers were prepared by diluting weighed samples of cyclodextrins with the amine-modified phosphate buffer. Enantiomers mixed at about 1:1 R:S were dissolved into 50% methanol/water (v/v). The concentration of the mixture was in the range 0.2–0.5 mg ml^{-1}. Buffers and sample solutions were filtered through 0.45 μm syringe filters (Gelman Scientific Acrodisc LC PVDF) prior to use.

2.3. Equipment and conditions

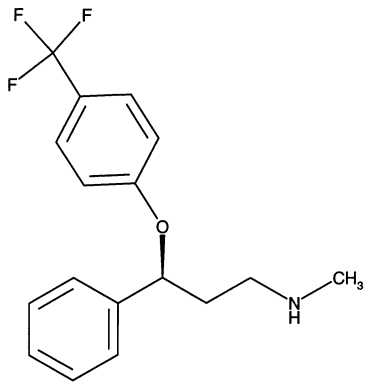
The work was performed on a P/ACE 5500 capillary electrophoresis instrument (Beckman Instruments, Fullerton, CA). The UV detector wavelength was 214 nm, and capillary temperature was 20°C. When using neutral cyclodextrin buffers, 30 kV was applied across a 50 μm I.D. × 57 cm bare silica capillary with normal polarity (detection at cathode). When using the sulfated-β-CD buffer, 12 kV was applied across a 25 μm I.D. × 27 cm bare silica capillary with reversed polarity. Sample solutions were introduced into the capillary by pressure (0.5 psi) for 5 s.

3. Screening method development

3.1. General considerations

A low pH buffer was selected to enhance solu-

Table 1
Structures of Lilly compounds



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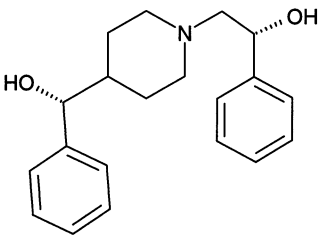

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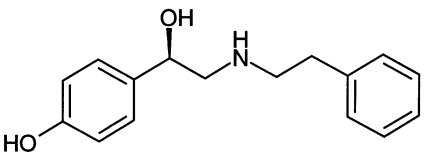

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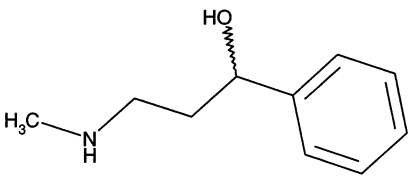

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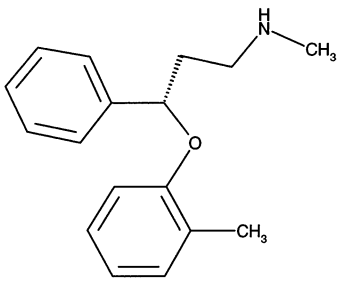

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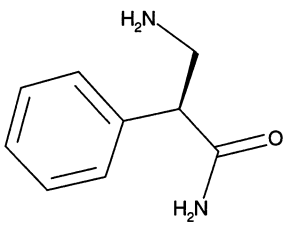

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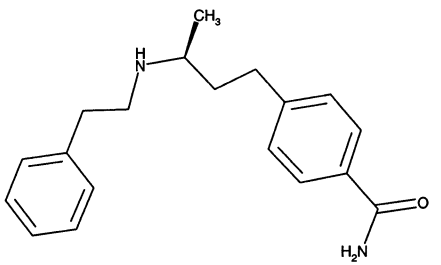

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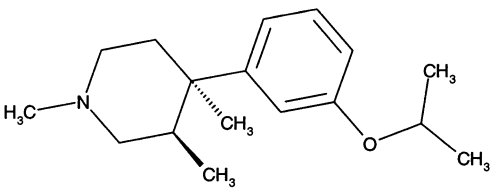

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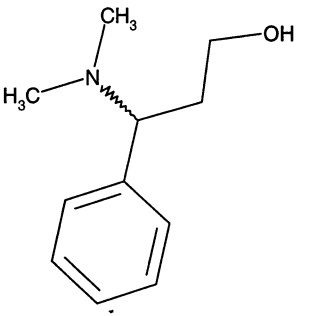

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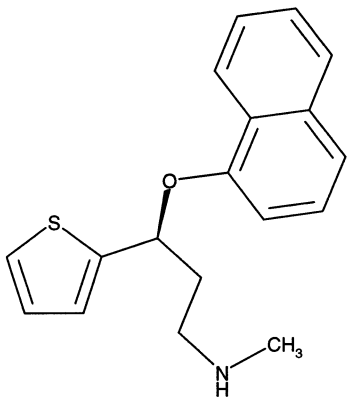

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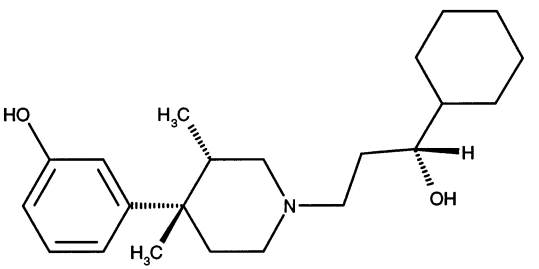

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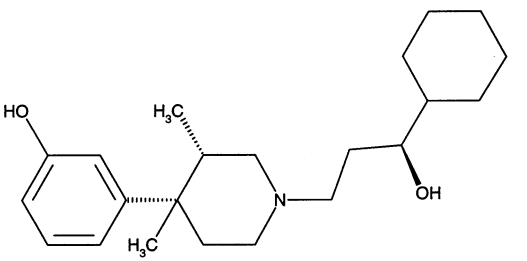

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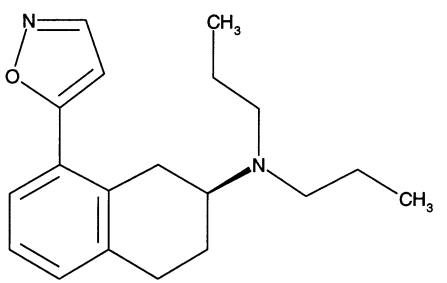

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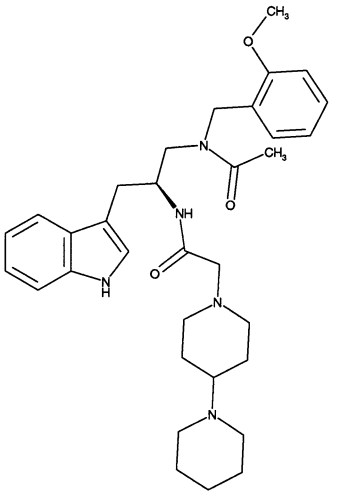

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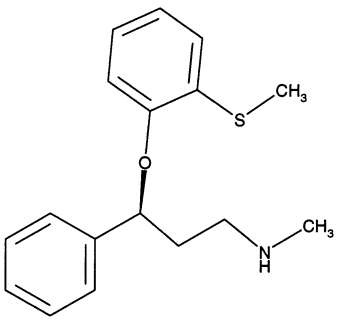

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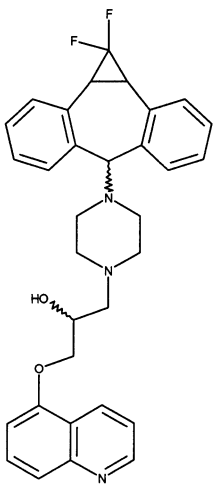

16, 17
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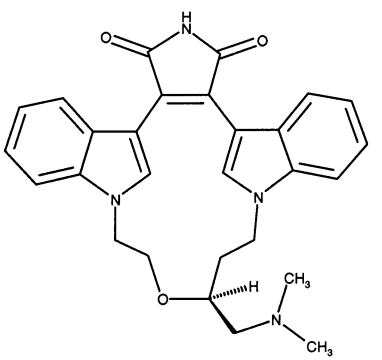

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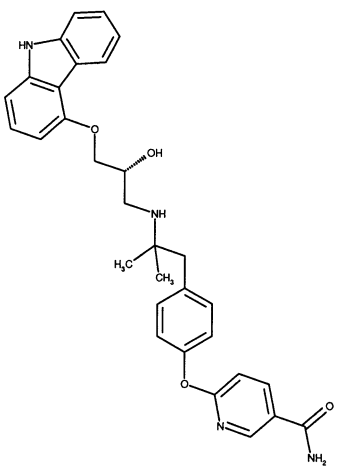

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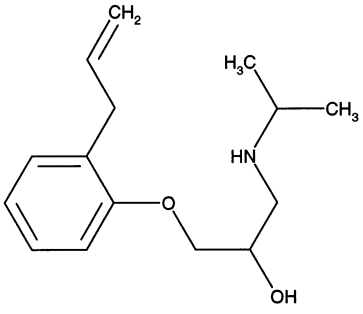
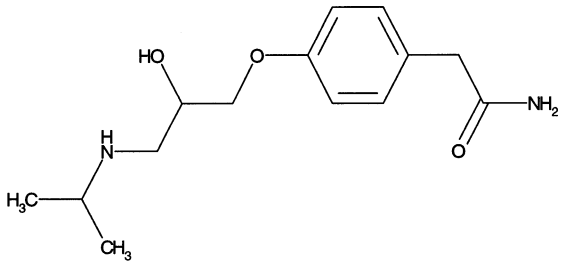
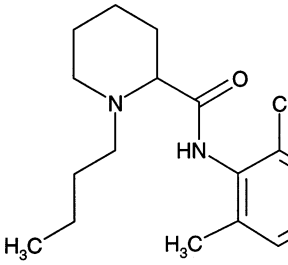
Table 2
Structures of commercially available compounds



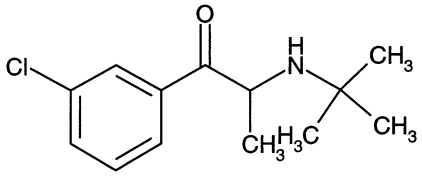
Alprenolol



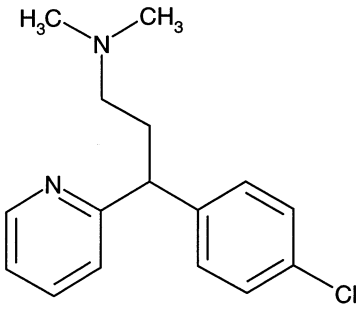
Atenolol



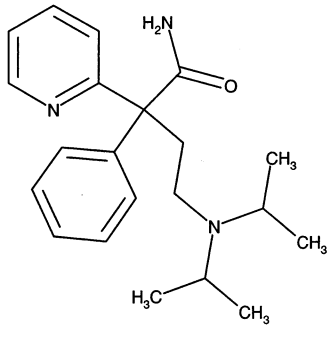
Bupivacaine



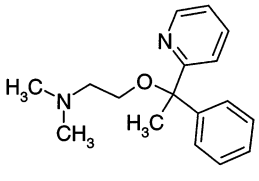
Bupropion



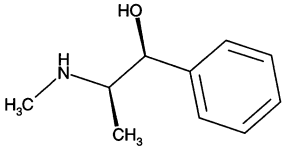
Chlorpheniramine



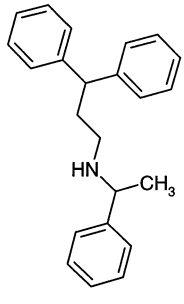
Disopyramide



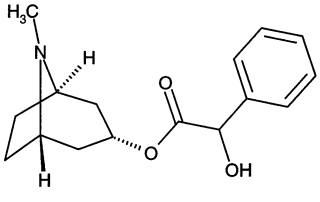
Doxylamine



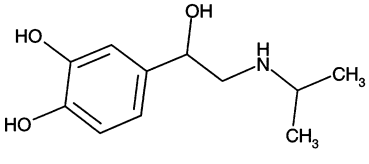
Ephedrine



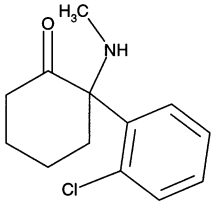
Fendiline



Homatropine



Isoproterenol



Ketamine

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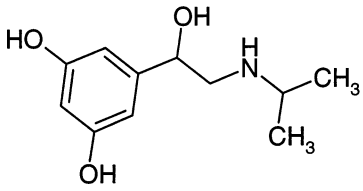
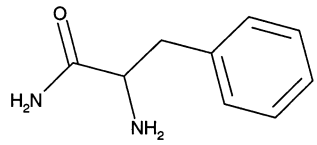
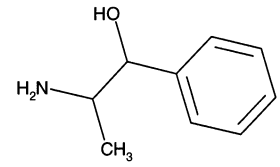


Metaproterenol



Phenylalaninamide



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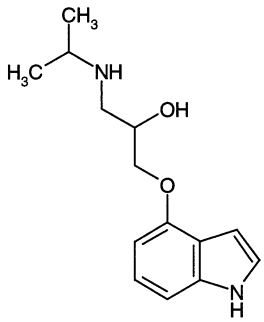
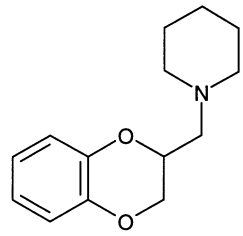
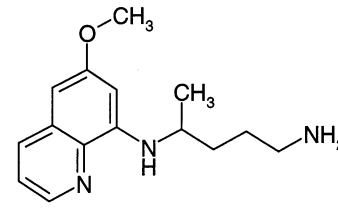


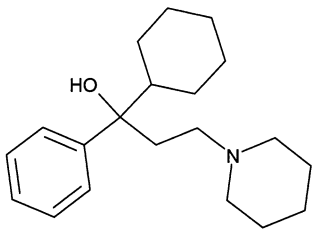
Pindolol



Piperoxan



Primaquine



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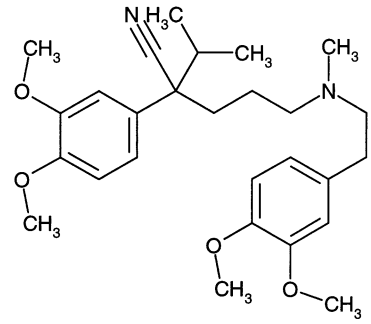
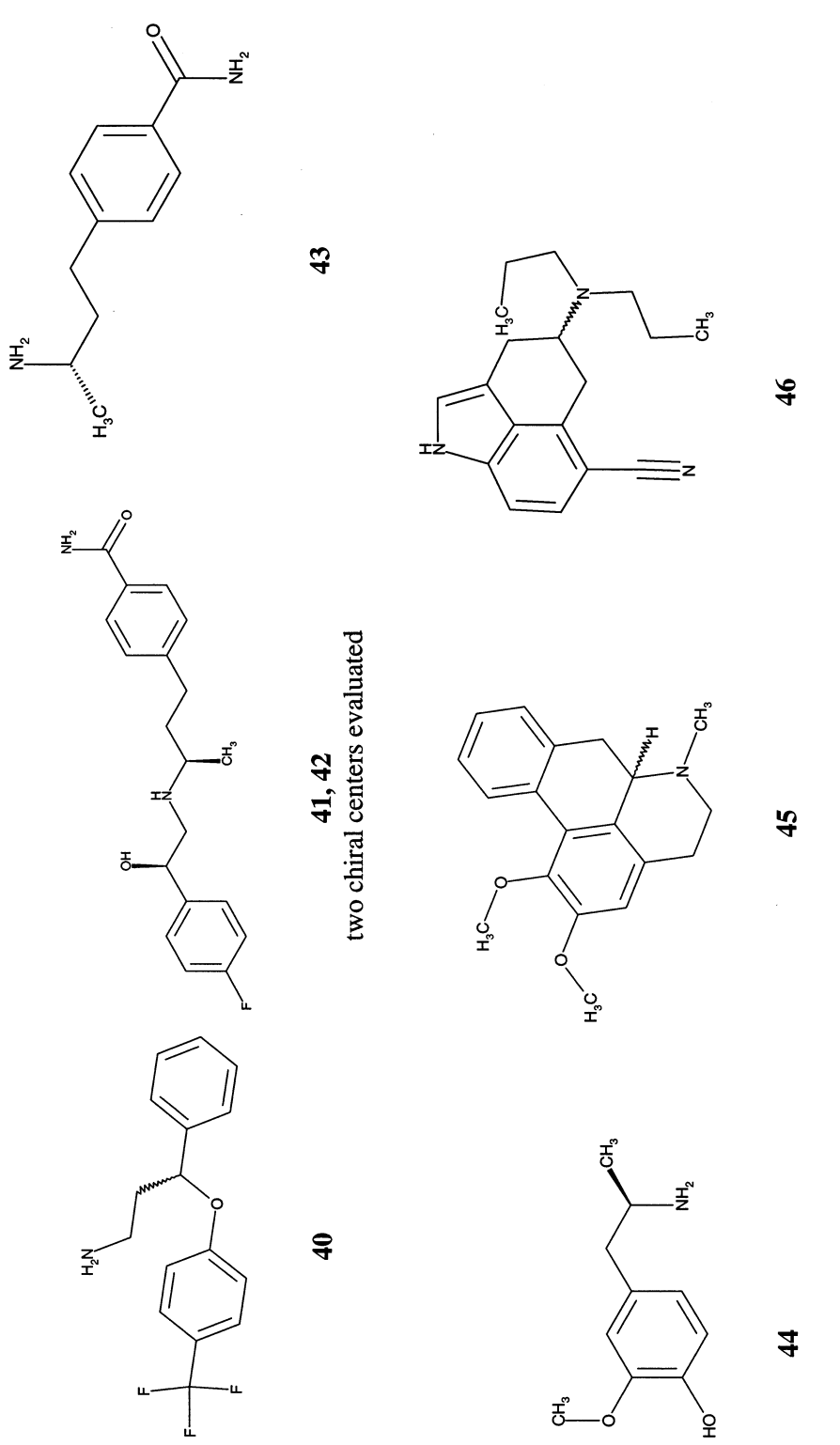


Verapamil

Table 3
Structures of Lilly compounds tested by final screening method (continued)



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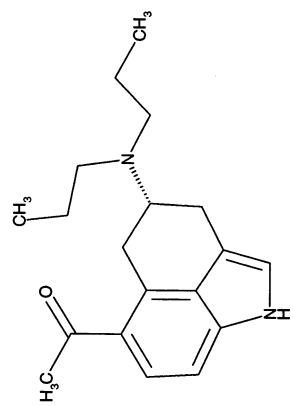
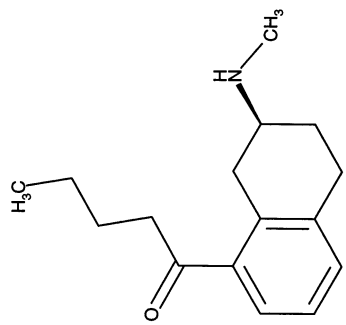
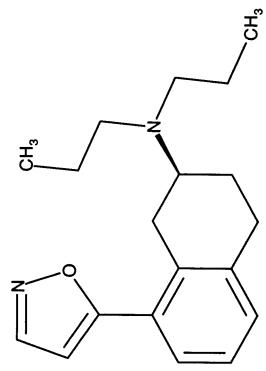
	**47**
	**48**
	**49**

Table 4
Chiral resolutions (R_S) obtained with a variety of cyclodextrins and concentrations (R_S value in bold type represents the best resolution for each compound)

ID #	Analyte	HP-β-CD		DM-β-CD		HP-α-CD		HP-γ-CD		Sul-β-CD
		5 mM	30 mM	5 mM	15 mM	5 mM	30 mM	5 mM	30 mM	32 mM
1	Fluoxetine	0.8	0	0.8	0	0	0	0	0.9	**2.1**
2	N/A	3.5	**4.4**	3.9	4.1	0.7	1.5	0	0.6	2.7
3	N/A	1.0	1.4	**2.0**	1.5	0	0	0	0	0.5
4	N/A	0.7	**2.8**	0.8	2.3	0	0.8	0	0	NP[1]
5	Tomoxetine	1.8	1.6	0.9	0.5	1.0	3.0	2.8	**3.5**	2.0
6	N/A	0	0.7	0	0.3	0	**1.0**	0	0	NP[a]
7	N/A	0	0	0	**0.9**	0	0	0	0.7	0
8	N/A	0	NP[a]	NP[a]	NP[a]	0	0	0	0	**1.1**
9	N/A	0	**1.0**	0	0.7	0	0	0	0	NP[a]
10	Duloxetine	2.9	0.8	1.2	0.6	3.4	**5.3**	0	0.3	4.9
11	N/A	NP[a]	NP[a]	NP[a]	NP[a]	0	0	0	0	**0.5**
12	N/A	NP[a]	NP[a]	NP[a]	NP[a]	0.3	0.4	0	0	**1.1**
13	N/A	0.6	**1.2**	0	0.8	0	**1.2**	0	0	0
14	N/A	**3.9**	1.0	0.7	0.7	0	0	0.7	0	1.6
15	N/A	2.4	1.9	1.9	1.2	3.7	**6.1**	4.2	5.7	3.7
16	N/A	0.6	0	**0.9**	0.7	0	0.8	0.7	0.5	0
17	N/A	0.7	0	0.7	0	0	0	**1.9**	0.6	0
18	N/A	0	0	0.2	0	0.3	**1.0**	0.6	**1.0**	NP[a]
19	N/A	**2.9**	0	0	0	0	0	**2.9**	1.2	0
20	Alprenolol	1.1	1.1	1.0	0.9	2.3	**2.8**	0	1.5	1.5
21	Atenolol	0	0.8	0.6	**1.1**	0	0	0	0	0
22	Bupivacaine	0	1.3	1.8	**3.9**	0	0	0	1.2	NP[a]
23	Bupropion	2.0	**2.2**	0.8	0.9	0	0	0	0	1.0
24	Chlorpheniramine	0.6	**2.7**	0	0.8	1.0	0	0	0	1.2
25	Disopyramide	0	0	0	**1.2**	0	0	0	0	NP[a]
26	Doxylamine	0	**0.6**	0	0	0.2	0	0	0	0
27	Ephedrine	0	1.3	0.7	**1.4**	0	1.0	0	0	0
28	Fendiline	NP[a]	NP[a]	NP[a]	0	0	0	**0.8**	0.7	0.6
29	Homatropine	2.7	**5.2**	2.3	2.8	0.1	1.7	0	0.8	3.5
30	Isoproterenol	1.7	**6.7**	3.9	6.2	0	0	0	0	4.0
31	Ketamine	0	1.0	1.6	1.6	0	1.3	0	1.3	**2.5**
32	Metaproterenol	3.3	3.4	**3.8**	3.4	0	0	0	0	1.9
33	Phenylalanimide	0	0	0	**0.8**	0	0.6	0	0	NP[a]
34	Phenylpropanolamine	0	0	0.7	**1.9**	0	0.7	0	0	0
35	Pindolol	0.6	1.2	0.9	**1.6**	0	0.7	0	0.7	0.6
36	Piperoxan	0	0	0.9	0.9	0	1.0	0	0.7	**1.4**
37	Primaquine	0	1.3	0.6	0.9	1.4	4.6	1.6	**11.0**	0.7
38	Trihexyphenidyl	0	0	0	0	**1.3**	1.1	0	0	1.2
39	Verapamil	0.4	0	0	0	0	0	0	0	**1.0**

[a] No peak eluted within 40 min.

bility and to generate mobility by protonating the amines. Sample mobility is necessary for use with the neutral cyclodextrin buffers.

The purpose of adding TBA or TEA to the buffers is to suppress the electroosmotic flow (EOF) in order to increase the enantioselectivity, and to eliminate analyte interactions with the uncoated capillary wall [3,6,8,9,18]. For the TBA-phosphate buffer used with neutral cyclodextrins, the EOF was measured to be -2.2×10^{-5} cm^2 V$^{-1} \cdot$ s^{-1} (negative sign represents the direction toward the anode). Under the same condition, the

Table 5
Percentage enantiomer pairs in the categories of $R_S > 0$, $R_S \geq 1$, $R_S \geq 1.4$, Best separation, and $t_m < 30$ min

% Enantiomers	HP-β-CD (%)		DM-β-CD (%)		HP-α-CD (%)		HP-γ-CD (%)		Sul-β-CD (%)
	5 mM	30 mM	5 mM	15 mM	5 mM	30 mM	5 mM	30 mM	32 mM
$R_S > 0$	51	59	62	72	31	51	23	46	59
$R_S \geq 1$	31	49	26	36	18	36	13	21	46
$R_S \geq 1.4$	23	26	21	28	10	18	13	10	31
Best separation	5.1	23	7.7	21	2.6	15	7.7	7.7	18
$t_m < 30$ min	85	72	79	77	97	95	100	87	82

typical electrophoretic mobility of basic compounds is $\sim 1 \times 10^{-4}$ cm^2 V$^{-1} \cdot$ s^{-1}. Therefore, basic compounds migrate toward the cathode even though the EOF is slightly reversed. The TEA-phosphate buffer used with the sulfated-β-CD had a weak, cathodic EOF of $\sim 1 \times 10^{-5}$ cm^2 V$^{-1} \cdot$ s^{-1}. Compared to neutral capillaries, a bare silica capillary under these conditions achieves the same goal of suppressing EOF, but is more rugged and economical.

Five modified cyclodextrins were selected: DM-β-CD, HP-β-CD, HP-α-CD, HP-γ-CD and Sul-β-CD. Among them, DM-β-CD and HP-β-CD have been widely used and are very effective chiral selectors based on literature data [3–9,14]. HP-α-CD and HP-γ-CD have higher separation rates than native CDs and their chiral selectivities are also more complementary to HP-β-CD and DM-β-CD [7]. Sul-β-CD has been successfully used for a large number of neutral and some basic compounds recently [19]. Incorporation of this charged CD was expected to give a selectivity complementary to the neutral cyclodextrins. Polarity was reversed with the Sul-β-CD buffer, due to its negative charge. Sulfated-gamma-cyclodextrin was also evaluated briefly as a potential chiral selector. However, the available degree of substitution was very high, such that excessive currents were generated. Therefore, this cyclodextrin was not pursued further.

In order to determine the impact of cyclodextrin concentration on resolution, low and a high concentrations were evaluated. Based on literature and experience, 5 mM was selected as an appropriate low concentration. Initially, 50 and 30 mM HP-β-CD were compared using eight chiral compounds, to help ascertain an appropriate high concentration for screening. Resolution was similar for the two concentrations, and run times were excessive in many cases at 50 mM. In order to maintain run times < 40 min and to preserve cyclodextrin supplies, 30 mM was selected as the high concentration of neutral cyclodextrins. For DM-β-CD, the concentration had to be lowered to 15 mM in order to maintain reasonably short run times. Comparisons were also made for Sul-β-CD at 5, 15, and 32 mM using 18 compounds. Little difference in resolution was found using these different concentrations. Therefore, only 32 mM was used for Sul-β-CD.

Voltages were selected to generate high field strengths to shorten the analysis time, while maintaining low currents (e.g. 20–30 μA for each of the final conditions).

The background noise and drift were found to be higher with Sul-β-CD buffer than with the neutral cyclodextrin buffers. The baseline improved slightly when the buffer was changed from phosphate/TBA to phosphate/TEA, and it significantly stabilized when a 25 μm capillary was used instead of 50 μm, due to the marked reduction of current.

3.2. Development approach

This paper focused on small basic compounds possessing chromophores and primarily with one or two chiral centers. A total of 20 commercially available and 18 Lilly compounds (Tables 1 and

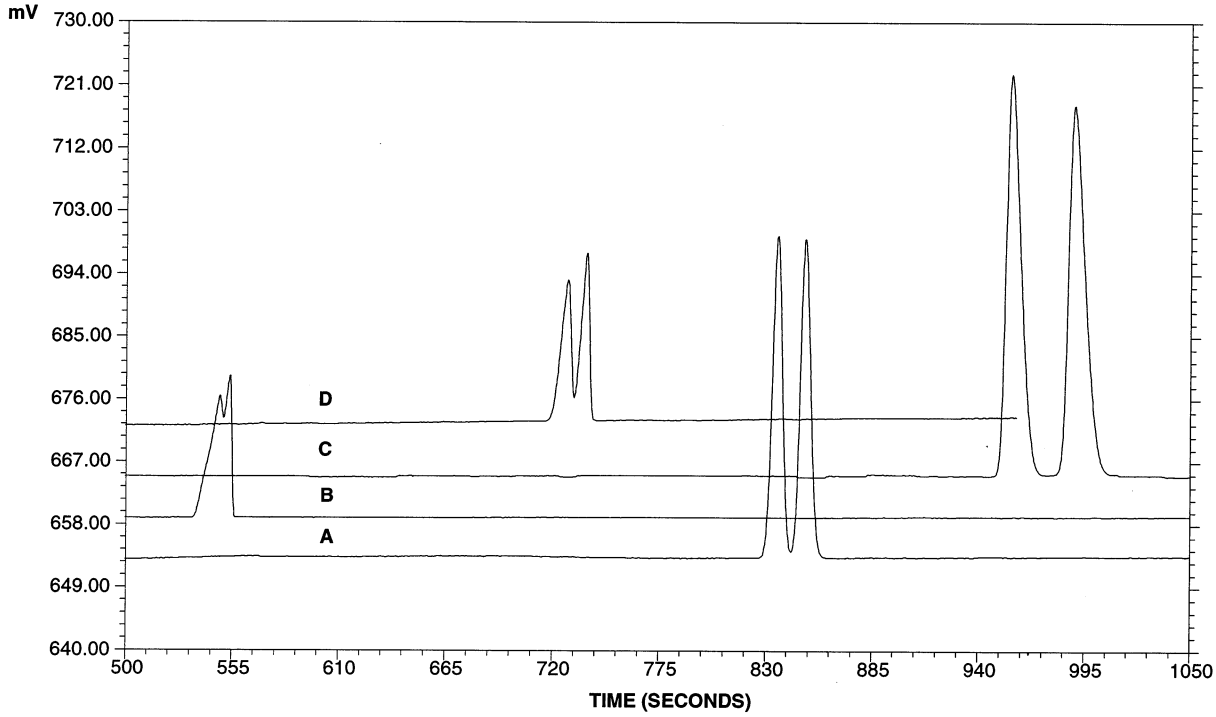


Fig. 1. Representative electropherograms using neutral CD buffers. Conditions: 30 mM phosphate/TBA (pH 2.5); 50 μm I.D. × 57 cm bare silica capillary, 30 kV, 20°C, 5 s injection of 1:1 R:S sample solution (0.2–0.5 mg ml^{-1} in 1:1 MeOH/water). (A) $R_S = 1.4$, ephedrine (15 mM DM-β-CD); (B) $R_S = 0.6$, chlorpheniramine (5 mM HP-β-CD); (C) $R_S = 2.0$, bupropion (5 mM HP-β-CD); (D) $R_S = 1.0$, compound ID # 9 (30 mM HP-β-CD).

2) were evaluated with the full set of cyclodextrin concentrations: 5 and 15 mM for DM-β-CD, 5 and 30 mM for the rest of the neutral cyclodextrins, and 32 mM for Sul-β-CD. The uncomplexed analyte migration time (t_m) was obtained by running the compounds in the pH 2.5 phosphate/TBA buffer without cyclodextrins. The most efficient screening strategy was determined based on the results and trends obtained. This strategy was then applied to an additional ten pairs of Lilly enantiomers (Table 3) to verify the validity of the method.

Note that the purpose of the paper is not to optimize the separation for each individual compound, but to provide a general screening tool that is fast and efficient.

4. Results and discussion

Table 4 lists the resolution values (R_S) obtained for 39 pairs of enantiomers studied in the screening method development. A value of zero indicates that no resolution was observed between the two enantiomers. A value of 1.4 indicates approximate baseline resolution, potentially adequate for quantitative analysis of the minor enantiomer [8]. The R_S value in bold type represents the best resolution for each pair. Note that partial or complete separation was obtained in every case, and over 50% of the compounds achieved approximately baseline separations. Table 5 summarizes the percentage of enantiomer pairs in the several categories: $R_S > 0$, $R_S \geq 1$, $R_S \geq 1.4$, Best separation, and $t_m < 30$ min. Figs. 1 and 2 show representative electropherograms obtained by neutral cyclodextrin buffers and sulfated-β-CD, respectively.

Our goal was to determine the most effective cyclodextrins based on their separation frequency

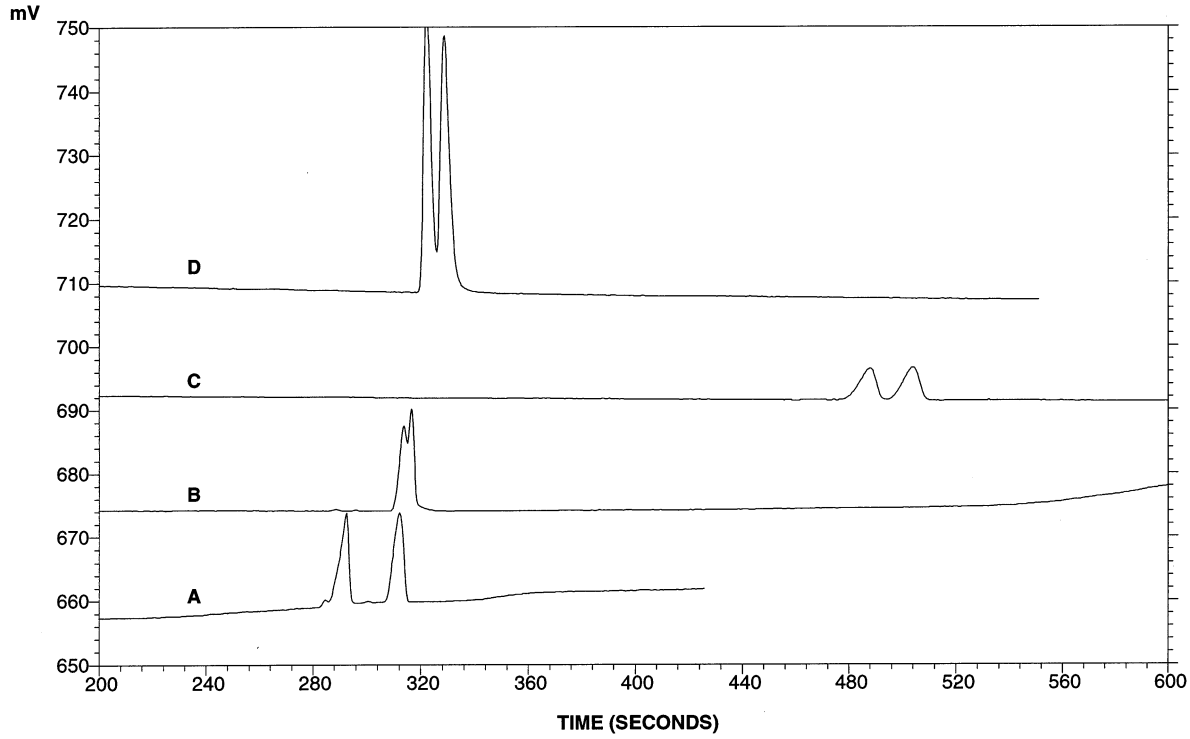


Fig. 2. Representative electropherograms using Sulfated-β-CD buffer. Conditions: 25 mM phosphate/TEA (pH 2.5) containing 5% (32 mM) Sul-β-CD; 25 μm I.D. × 27 cm bare silica capillary, 12 kV, 20°C, 5 s injection of 1:1 R:S sample solution (0.2–0.5 mg ml^{-1} in 1:1 MeOH/water). (A) $R_S = 2.0$, compound ID # 5; (B) $R_S = 0.5$, compound ID # 3; (C) $R_S = 1.5$, Alprenolol; (D) $R_S = 1.0$, Verapamil.

and complementary selectivity. As shown in Tables 4 and 5, 15 mM DM-β-CD separated the largest number of compounds. A concentration of 30 mM HP-β-CD and Sul-β-CD separated the most compounds with $R_S \geq 1$ or $R_S \geq 1.4$, respectively. The above three cyclodextrins also showed different selectivities. For 31% of the analytes (ID # 5, 7, 16, bupivacaine, bupropion, chlorpheniramine, disopyramide, doxylamine, homatropine, phenylalanimide, phenylpropanolamine, and piperoxan), DM-β-CD and HP-β-CD had differences in resolution of greater than 1, or one produced a separation when the other one did not. Using Sul-β-CD, significantly better separations were obtained over neutral cyclodextrins in 18% of the cases (ID # 1, 8, 11, 12, ketamine, piperoxan, and verapamil). Generally, more compounds were separated and better resolutions were obtained at higher CD concentration (in the concentration range of this study) with only a few exceptions. The data also showed that HP-α-CD and HP-γ-CD were complementary to DM-β-CD and HP-β-CD, although they did not produce as many separations. 26% of the enantiomer pairs were separated best with HP-α-CD or HP-γ-CD (ID # 5, 6, 10, 15, 17, 18, alprenolol, fendiline, primaquine and trihexyphendinyl). It is interesting to note that for several of the compounds (ID # 5, 15, 18, and 37), HP-α-CD and HP-γ-CD both yielded better resolution than HP-β-CD. This implies that the complexes formed by HP-α-CD and HP-γ-CD in these cases may differ from each other in the sites of interaction on the analyte and/or CD.

Table 6 compares our data to those published using neutral capillaries. The migration factor (t_2/t_1) is used since the data from the literature

Table 6
Migration factor (t_2/t_1) comparison[a] between neutral capillary separations [7] and this work (bold, italics)

Analyte	HP-β-CD	DM-β-CD	HP-α-CD	HP-γ-CD
Alprenolol	1.018	1.000	1.035	1.019
	1.023	***1.020***	***1.051***	***1.019***
Atenolol	1.012	1.000	1.000	1.000
	1.016	***1.016***	***1.000***	***1.000***
Bupivacaine	1.009	1.049	1.011	1.021
	1.018	***1.055***	***1.000***	***1.000***
Chlorpheniramine	1.000	1.000	1.021	1.000
	1.034	***1.007***	***1.000***	***1.000***
Disopyramide	1.000	1.017	1.000	1.000
	1.000	***1.015***	***1.000***	***1.000***
Doxylamine	1.000	1.000	1.035	1.000
	1.015	***1.000***	***1.000***	***1.000***
Fendiline	1.000	1.000	1.000	1.013
	1.000	***1.000***	***1.000***	***1.019***
Homatropine	1.086	1.067	1.013	1.000
	1.141	***1.074***	***1.028***	***1.015***
Ketamine	1.012	1.019	1.032	1.018
	1.016	***1.041***	***1.021***	***1.015***
Pindolol	1.019	1.021	1.022	1.010
	1.021	***1.024***	***1.008***	***1.010***
Trihexyphenidyl	1.000	1.000	1.014	1.000
	1.000	***1.000***	***1.024***	***1.000***

[a] Literature conditions [7]: 0.1 M monobasic sodium phosphate adjusted to pH 2.5 with phosphoric acid, containing 45 mM cyclodextrin; 50 μm I.D. × 30 cm polyacrylamide-coated fused silica capillary; 15 kV; capillary temperature: 25°C; sample temperature: 20°C; 15 kV electrokinetic injection for 3 s; 200 nm detection.

were expressed this way. The migration times of the first and second eluted enantiomers are given by t_1 and t_2. Our results are equally good, if not better, than those obtained with neutral capillaries [4,5,7].

Although no attempt was made to optimize any separations, the results compared favorably to optimized results reported in the literature [3] (Table 7).

Table 7
Comparison of the best resolution obtained in this work (not optimized) with the literature data (optimized)

Analyte	R_s (this work)	R_s (literature)
Alprenolol	2.8	1.4
Atenolol	1.1	1.4
Pindolol	1.6	1.4
Bupivacaine	3.9	6.0
Chlorpheniramine	2.7	2.8
Ephedrine	1.4	2.7

4.1. Proposed screening method

Based on the above results, the following screening method is proposed using bare silica capillaries at 20°C, 214 nm, and the following run buffers in the order listed:

1. 30 mM phosphate/TBA, pH 2.5 (Uncomplexed reference)
2. 15 mM DM-β-CD/30 mM phosphate/TBA (pH 2.5)
3. 30 mM HP-β-CD/30 mM phosphate/TBA (pH 2.5)
4. 30 mM HP-α-CD/30 mM phosphate/TBA (pH 2.5)
5. 30 mM HP-γ-CD/30 mM phosphate/TBA (pH 2.5)
6. 32 mM Sul-β-CD/25 mM phosphate/TEA (pH 2.5)

In steps 1–5, 30 kV may be applied using a 50 μm I.D. × 57 cm bare silica capillary with normal polarity (detector at cathode). In step 6, 12 kV

Table 8
Chiral resolution of test compounds using the proposed screening method

ID #	DM-β-CD 15 mM	HP-β-CD 30 mM	HP-α-CD 30 mM	HP-γ-CD 30 mM	Sul-β-CD 5%
40	0.6	0	0	1.2	1.6
41	NP[a]	1.1	ND[b]	ND[b]	0
42	NP[a]	0.9	ND[b]	ND[b]	0
43	0	0	0	0	0
44	0	0.5	0	0.8	NP[a]
45	0	0	0	0	0
46	0	1.9	N/A[c]	N/A[c]	N/A[c]
47	0	3.1	N/A[c]	N/A[c]	N/A[c]
48	1.5	1.4	N/A[c]	N/A[c]	N/A[c]
49	0.8	1.3	1.3	0.23	0.1

[a] No peak eluted within 40 min.
[b] Not determined due to the overlap of the two pair of peaks in the mixture (containing two chiral centers), which caused difficulty in determination of the peak identity.
[c] Not attempted since baseline separation was already attained.

may be applied using a 25 μm I.D. × 27 cm bare silica capillary with reversed polarity.

Sul-β-CD buffer provides complementary selectivity and generally shorter run times compared to the neutral cyclodextrin buffers. However, due to the less stable baseline, and the need to change buffer, capillaries, and polarity, this buffer is used as a final selection.

4.2. Results from the test samples

The proposed screening method was applied to some additional Lilly compounds, and the results are listed in Table 8.

80% (8/10) of the compounds were separated, and 50% (5/10) achieved nearly baseline separation or better. The proposed screening strategy proved to be an efficient tool in rapidly identifying separation conditions.

5. Conclusions

A fast screening CE separation method is proposed for small, amine-containing enantiomers possessing chromophores and primarily with one or two chiral centers. The method utilizes bare silica capillaries and pH 2.5 TBA or TEA phosphate buffer containing modified cyclodextrins. Thirty-nine pairs of enantiomers were used for developing the screening method, and an additional ten compounds were tested for the method validity. Although the method is not intended to optimize the separations, in several cases we obtained results comparable to the available literature data obtained under optimal conditions. Overall, partial or complete separation was achieved in 96% of the cases (47/49), and 55% of the enantiomer pairs (27/49) gave resolutions ≥ 1.4.

As a screening approach, the method includes only the limited types of cyclodextrins considered to be the most effective. Separations not achieved by this screening method may still be possible using different cyclodextrins or other chiral selectors, or under different operating conditions. Optimization for each individual compound, if necessary, is another step, not covered in this study.

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