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Enantiomeric resolution of primary amines by capillary electrophoresis and high-performance liquid chromatography using chiral crown ethers

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Abstract

High-performance liquid chromatography with a chiral crown ether stationary phase and capillary electrophoresis (CE) with a chiral crown ether dissolved in the operating buffer have been used for the separations of enantiomers of a variety of primary amines of pharmaceutical interest, including aminotetralin analogues, aminomethylbenzodioxane, amino derivatives of naphthalene and phenanthrene, and aminodecalin analogues. Interestingly, the enantiomers of many of these compounds were adequately resolved by only one or the other of the two methods, indicating that the techniques are complementary. The influence of the degree of complexation of analyte molecules with the crown ether on chromatographic retention, electrophoretic migration, and chiral recognition is discussed, as well as the relative advantages and disadvantages of the two methods in practical applications.

1. Introduction

High-performance liquid chromatography (HPLC) using various types of chiral stationary phases, mobile phase additives, and derivatizing agents has been used extensively for the analysis of amino acids and amines [1,2]. More recently, capillary electrophoresis (CE) has been applied to chiral separations [3]. Although some work has focused on the use of CE for the separation of diastereomeric derivatives of chiral compounds, much of the work has focused on the use of chiral selectors in the electrophoretic operating buffer. Most commonly, cyclodextrin buffer additives are used for chiral separations by CE. More recently, Kuhn and co-workers [4,5] described the use of a chiral crown ether for chiral resolution of various amino acids, peptides, and optically active amines without prior derivatization. In this work we compare the use of CE with a chiral crown ether in the buffer and HPLC with a crown ether stationary phase for the enantiomeric resolution of primary amines of pharmaceutical interest.

2. Experimental

All CE experiments were carried out using a Beckman P/ACE 2100 CE system. The system was interfaced to an IBM-compatible personal computer and Beckman System Gold software was used for data collection and manipulation. Separations were carried out in a fused-silica

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capillary tube (Beckman Instruments, Neuilly, France). The dimensions of the tube were 57 cm × 75 μm I.D., with the detector located 7 cm from the outlet end of the capillary. A detector slit size of 100 μm × 200 μm was used for experiments with direct detection. The detection wavelength was either 254 or 280 nm, depending on the absorbance spectrum of the analyte. The capillary was thermostatted at 25°C. Most experiments were carried out using a 50 m*M* sodium phosphate buffer at pH 2.2 containing 30 m*M* 18-crown-6 tetracarboxylic acid (18-crown-6 TCA). Sample concentrations were typically 0.5 mg/ml in water. Pressure injections of 2–3 s (approximately 10–15 nl) were made.

For chiral resolutions of compounds which have poor UV absorbance, an electrolyte solution containing 6 m*M* benzyltrimethylammonium chloride (BTMACl) and 30 m*M* 18-crown-6 TCA and the pH adjusted to 3.7 with 1 *M* NaOH was used, with indirect detection. The detector wavelength was set at 214 nm. A slit size of 50 μm × 200 μm was used for measurements with indirect detection. A 57 cm × 75 μm I.D. capillary thermostatted at 25°C was used for these experiments. Sample concentrations were typically 0.5 mg/ml in water. Pressure injections of 5 s (approximately 26 nl) were made.

HPLC experiments were carried out using a conventional HPLC system consisting of a Waters M590 pump, a Waters WISP 710B autosampler, and either a Waters M481 UV detector or a Kratos Spectroflow 773 UV detector. A CrownPak(+) column (15 cm × 4 mm I.D., 5 μm packing) obtained from Daicel (Tokyo, Japan) was used. The column was thermostatted using either a water bath or a methanol bath. A guard column (2.1 cm × 2.0 mm I.D.) containing glass beads (30–50 μm diameter) was used. For those experiments using post-column derivatization with *o*-phthalaldehyde (OPA) and fluorescence detection, a Dosapro minipump from Milton Roy was used to pump the derivatization reagent. A Shimadzu RF530 detector was used, with an excitation wavelength of 360 nm and emission wavelength of 450 nm. The conditions of the OPA derivatization were as described in Ref. 6, except that the ionic strength of the potassium borate buffer used for pH control in these experiments was higher (0.8 *M*). Unless otherwise indicated, the following chromatographic conditions were used for the aminotetralin analogues: mobile phase, 0.013 *M* $HClO_4$, pH 2.04, 15% methanol; temperature, 40°C; flow-rate, 1 ml/min; detector wavelength, 210 nm. The conditions used for the aminonaphthalene analogues were: mobile phase, 0.16 *M* $HClO_4$, pH 1.00, 15% methanol; temperature, 30°C; flow-rate, 1 ml/min; detector wavelength, 254 nm. The conditions used for the aminophenanthrene analogues were, unless otherwise stated: mobile phase, 0.0011 *M* $HClO_4$, pH 2.92, 15% methanol; temperature, 30°C; flow-rate, 1 ml/min; detector wavelength, 280 nm. The chromatographic conditions used for the aminodecalin analogues were: 0.16 *M* $HClO_4$, pH 0.98, 15% methanol; temperature, 30°C; flow-rate, 1 ml/min; detector wavelength; 210 nm. Sample concentrations for all experiments were typically 0.5 mg/ml in water or eluent and injections volumes were 10 μl.

Chemicals were reagent grade, unless otherwise stated. Phosphoric acid, sodium hydroxide, perchloric acid and methanol were obtained from Merck (Darmstadt, Germany). 18-Crown-6 TCA was also obtained from Merck. BTMACl was obtained from ICN Pharmaceuticals (Plainview, NY, USA). Water was purified from laboratory water using a Millipore water-purification system. 1-Aminotetrahydronaphthalene was obtained from Aldrich (Saint Quentin Fallavier, France) The other aminotetralins and aminomethylbenzodioxane were synthesized in our laboratories by published procedures [7,8]. The amino derivatives of naphthalene and phenanthrene were prepared as described in Ref. 9. The 3-amino-2-decalones [10,11] and decalylamine [12] were also prepared in our laboratories.

3. Results and discussion

3.1. Aminotetralins

We investigated a group of aminotetralins, including positional isomers and substituted ana-

logues. Rondelli et al. [13] reported the enantiomeric resolution of a substituted aminotetralin by HPLC, following chiral derivatization with *R*-(+)-α-methylbenzylisocyanate. Witte et al. [14] have done an extensive study of resolution of substituted aminotetralins by HPLC using a cellulose tris-3,5-carbamate stationary phase, but most of the compounds in this study were secondary or tertiary amines. The HPLC retention times, CE migration times, separation factors (α), and resolution (R_s) for the aminotetralins and aminomethylbenzodioxane are given in Table 1. The separation factor in HPLC was calculated as described in Ref. 15. The separation factor for CE was calculated as indicated in Ref. 5. Resolution for both methods was calculated according to Ref. 15.

Structures of the crown ethers used in the HPLC and CE systems are shown in Fig. 1. In the CE system, there are two mechanisms for chiral recognition. These have been previously discussed by Kuhn et al. [5]. Recognition occurs by either a steric barrier mechanism or by hydrogen bonding between the guest molecule and the carboxylic acid groups on the crown

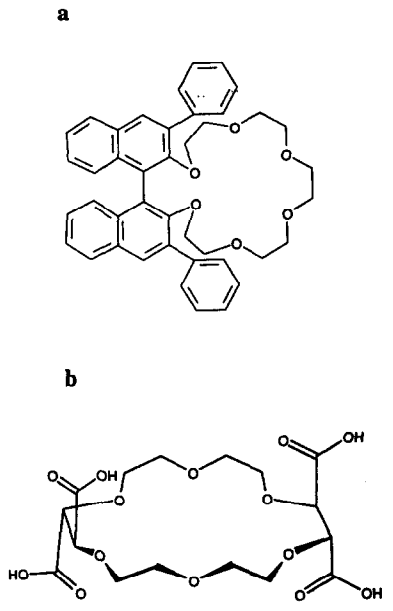
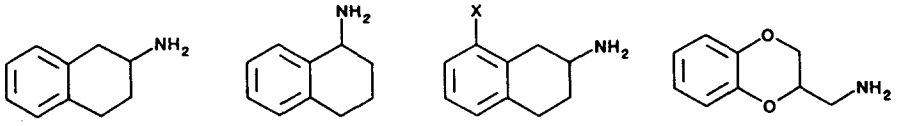


Fig. 1. (a) (*S*)-2,3:4,5-Bis(1,2,3-phenylnaphtho)-1,6,9,12,15,18-hexaoxacycloeicosa-2,4-diene, crown ether incorporated into the stationary phase of the CrownPak(+) HPLC column. (b) (2*R*,3*R*,11*R*,12*R*)-(+)-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxylic acid, crown ether used in the CE operating buffer.

Table 1
Results for aminotetralins and aminomethylbenzodioxane



Compound 1 Compound 2 Compounds 3 - 5 Compound 6

Compound	X	HPLC Results				CE Results			
		t_{R1}	t_{R2}	α	R_s	t_{m1}	t_{m2}	α	R_s
1		9.01	11.53	1.31	2.82	20.56		1.000	0
2		4.61	5.60	1.27	1.27	13.22	17.86	1.351	3.34
3	OCH_3	23.96	28.90	1.21	1.88	17.72	17.91	1.011	0.41
4	C≡N	18.82	19.91	1.06	0.51	20.91	22.94	1.097	6.12
5	Br	–	–	–	–	20.93	22.07	1.054	3.68
6		7.41	11.59	1.56	3.45	24.95	25.45	1.020	1.12

t_{R1}, t_{R2} = HPLC retention times (min) of the first- and second-eluting isomers, respectively; t_{m1}, t_{m2} = CE migration times (min) of the faster- and slower-migrating isomers, respectively.

ether. Similarly, chiral recognition in the HPLC system occurs by either a steric barrier mechanism or through hydrophobic interactions between the guest molecule and the hydrophobic substituents on the crown ether incorporated into the HPLC stationary phase.

The results for compounds **1** and **2** indicate that the position of the amino group is important in chiral recognition for these compounds. By HPLC, 2-aminotetralin (compound **1**) is more strongly retained and better resolved than 1-aminotetralin (compound **2**). In the CE experiment, the opposite result was observed. Compound **1** exhibits a strong affinity for the crown ether in the electrophoresis buffer, as evidenced by its longer migration time, but no chiral resolution is observed. However, compound **2**, while more weakly complexed by the crown ether, is well resolved. The geometry of compound **2** must lead to a larger difference in the stability constants for its enantiomers with the crown ether as compared to its positional isomer, compound **1**. A large number of spikes were observed in the electropherograms for compounds **1** and **2**, indicating that there may be a problem with the solubility of these compounds in the operating buffer.

Results for compounds **3–5** demonstrate the effect of different substituents at the 9-position on chiral resolution for substituted 2-aminotetralins. In the HPLC system, if non-stereospecific interactions such as hydrophobic interactions with the stationary phase are weak, then the retention time can be used as an indicator of the degree of complexation of the analytes with the crown ether. In the CE system, other factors contribute to the overall migration rate of an analyte, such as the molecular size and shape. Complexation with the crown ether effectively retards the migration of analytes in this system. For a group of analytes with similar size and charge, the migration time in CE will be a rough indicator of the degree of complexation. In Fig. 2, the separation factor, α, is plotted as a function of the retention time of the first-eluting isomer in HPLC and the migration time of the first-eluting isomer in CE. These plots indicate that in both systems there is a correlation between the strength of complexation, as measured by the migration time for CE and the retention time for HPLC, and the degree of chiral recognition, as measured by α. Interestingly, we observed that for those compounds which were well resolved by CE (compounds **4** and **5**), poor results were obtained by HPLC. Conversely, compound **3** was well resolved by HPLC, by not by CE. Compound **5** was well resolved by CE, but was not eluted from the CrownPak(+) column. We found no conditions that would elute this compound from the column. No indications of solubility problems, such as spurious spikes, were observed in the electropherograms for compounds **3–5**.

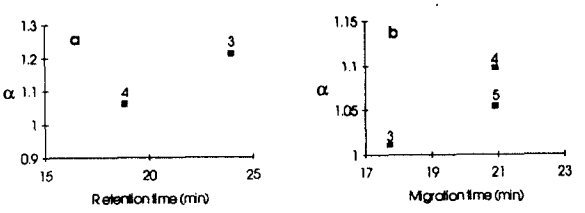


Fig. 2. (a) Correlation between separation factor, α, and HPLC retention time. (b) Correlation between α and CE migration time. Compound numbers refer to Table 1.

One compound with the amine group located two carbons from the chiral center (compound **6**) was also included in this study. This compound was very well resolved by HPLC, most likely because the position of the aromatic portion of the guest molecule with respect to the amino group permits hydrophobic interactions with the aromatic portion of the crown ether in the stationary phase of the CrownPak(+) column. Compound **6** is also adequately resolved by CE. The resolution is less than that observed by HPLC since there is no possibility of hydrophobic interactions assisting in chiral recognition. However, this data is in contrast to results observed by Kuhn et al. [5] in which compounds with the chiral center a the β-carbon were poorly resolved by CE under conditions similar to ours. Kuhn et al. observed better selectivity by CE for compounds with bulky substituents, due to increased steric hindrance. Compound **6** is bulkier than the compounds in Kuhn et al.'s study and thus a better separation is obtained. Representa-

tive chromatograms of compounds **4** and **6** are given in Fig. 3. Corresponding electropherograms are shown in Fig. 4.

3.2. Amino derivatives of naphthalene and phenanthrene

The results for a group of bicyclic and tricyclic aromatic amines are summarized in Table 2. This group of compounds can be divided into two sub-groups, aminonaphthalene analogues (compounds **7–10**) and aminophenanthrene analogues (compounds **11–15**). For the aminonaphthalene analogues (compounds **7–10**), the effect of substitution on chiral recognition by the crown ethers is apparent. Two methyl groups at the

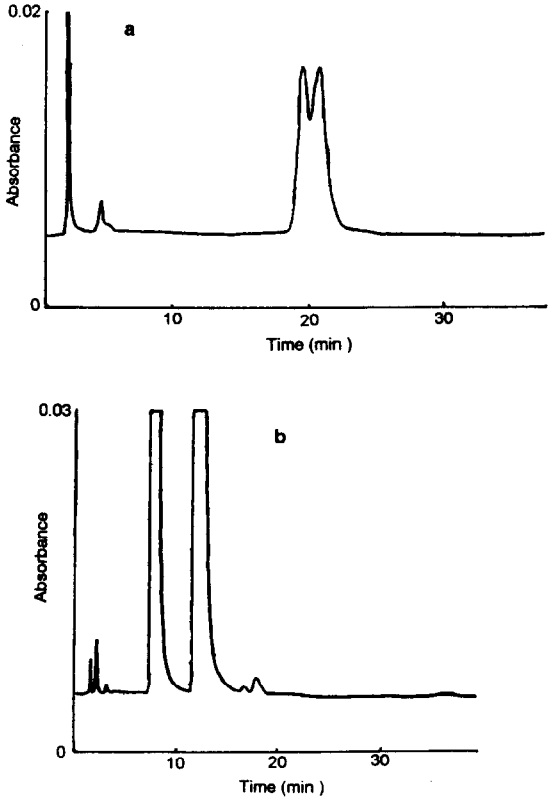


Fig. 3. Chromatograms of (a) compound **4**, 162 ng injected; and (b) compound **6**, 5.7 μg injected. Conditions: Column, CrownPak(+), 15 cm × 4.0 mm; mobile phase, 0.013 *M* $HClO_4$, pH 2.04, 15% methanol; temperature, 40°C; flow-rate, 1 ml/min; detector wavelength, 210 nm; injection volume, 10 μl.

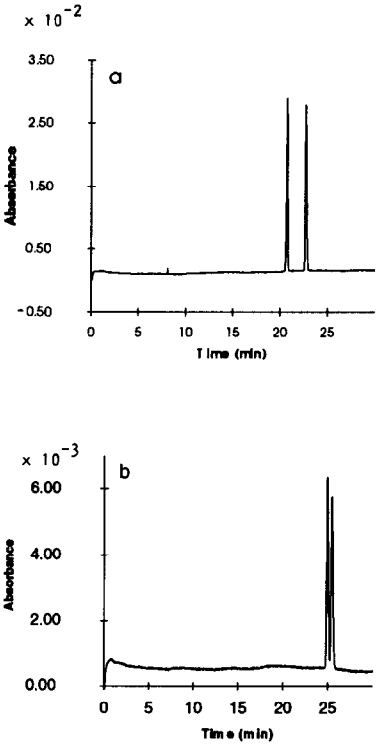


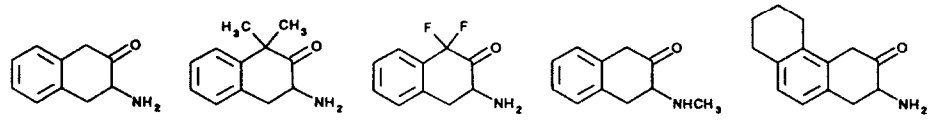
Fig. 4. Electropherograms of (a) compound **4**, 7.5 ng injected; and (b) compound **6**, 5 ng injected. Conditions: capillary, 57 cm × 75 μm I.D.; buffer, 50 m*M* sodium phosphate, pH 2.2, 30 m*M* 18-crown-6 TCA; temperature, 25°C; voltage, +15 000 V; detector wavelength, 210 nm.

3-position (compound **8**) result in a large increase in retention and resolution by HPLC. Similarly, dimethyl substitution at the 3-position results in increased migration times and resolution in the CE experiment. The substitution of two fluorine atoms at the 3-position (compound **9**) results in decreased retention and resolution by HPLC. But in the CE experiment, increases in migration time and resolution were observed.

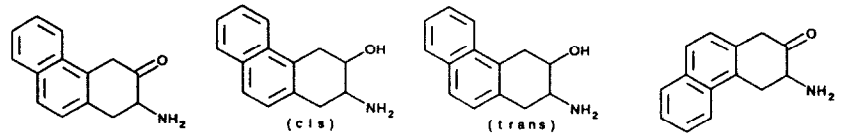
In Fig. 5, the separation factor, α, is plotted versus retention time in HPLC and migration time in CE for compounds **7–10**. The plots indicate that for this group of compounds, there is a correlation between the degree of complexation and the degree of chiral recognition in both systems.

Compound **10** is a secondary amine which does not form complexes with crown ethers. As

Table 2
Results for aminonaphthalenes and aminophenanthrenes



Compound 7 Compound 8 Compound 9 Compound 10 Compound 11



Compound 12 Compound 13 Compound 14 Compound 15

Compound	Eluent	HPLC Results				CE Results			
		t_{R1}	t_{R2}	α	R_s	t_{m1}	t_{m2}	α	R_s
7	A	8.46	16.67	2.16	3.40	18.89	20.76	1.099	1.89
8	A	16.61	38.61	2.45	9.00	25.19	28.34	1.125	4.88
9	A	5.61	6.47	1.20	1.46	19.68	21.73	1.104	2.22
10	A	4.02	–	1.00	0	14.61	–	1.000	0
11	B	47.54	80.18	1.70	2.52	18.00	18.96	1.053	1.07
12	B	31.77	68.61	2.26	3.00	18.50	19.23	1.039	3.00
13	B	17.71	22.75	1.31	1.03	20.87	21.14	1.013	0.73
14	B	31.38	58.07	1.89	2.58	16.64	17.72	1.065	3.36
15	C	37.5	71.4	1.96	1.28	19.84	20.07	1.011	<0.5

Eluents: (A) 0.16 *M* $HClO_4$, pH 1.00, 15% methanol; (B) 0.0011 *M* $HClO_4$, pH 2.92, 15% methanol; (C) 0.0016 *M* $HClO_4$, pH 2.51, 15% methanol. Other experimental conditions given in text. t_{R1}, t_{R2}, t_{m1} and t_{m2} as in Table 1.

expected, the HPLC retention time and the CE migration time are shorter for this molecule than for its demethylated analogue (compound **7**). Also, no chiral resolution was achieved for this compound, which shows that complex formation is required for chiral recognition. Similar results were observed previously by Kuhn et al. [5].

The second sub-group, aminophenanthrene analogues (compounds **11–15**), have three fused rings and are significantly more hydrophobic than the aminonaphthalene analogues. For these compounds, the relationship between HPLC retention time and chiral recognition is less clear (see Fig. 6a). This is probably due to the increased hydrophobicity of these compounds in

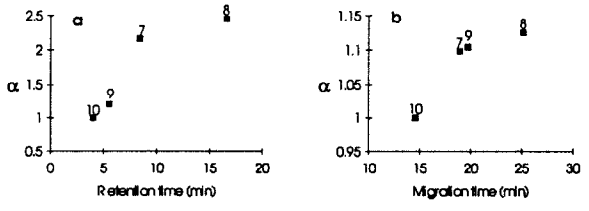


Fig. 5. (a) Correlation between separation factor, α, and HPLC retention time. (b) Correlation between α and CE migration time. Compound numbers refer to Table 2.

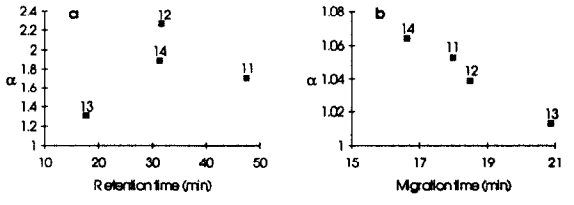


Fig. 6. (a) Correlation between separation factor, α, and HPLC retention time. (b) Correlation between α and CE migration time. Compound numbers refer to Table 2.

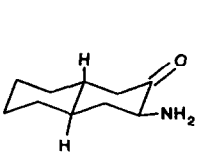
comparison to those having only two rings. Increased hydrophobicity contributes to retention, but not to chiral resolution. Thus retention time is no longer a good indication of the degree of complexation in the HPLC system. The chromatographic retention times for most of these compounds were extremely long as 60–80 min in most cases, and the peaks were quite broad. By CE, analysis times are fairly short in comparison to HPLC. Because the peaks in CE are very narrow, good resolution is still achieved for three of the five compounds. The relationship between the CE migration time and the observed separation factor is unclear (see Fig. 6b). This indicates that factors such as molecular size and shape or perhaps a difference in the degree of ionization, are at least as important as complexation with the crown ether in the overall mobilities of the analytes.

For this series of compounds, the structure of the molecule and steric effects leading to a large difference in stability constant appear important for chiral recognition in both the HPLC and CE systems. For example, for compounds **13** and **14**, which are *cis/trans* isomers, the *trans* isomer is better resolved in both the HPLC and CE systems. The much shorter retention times by HPLC indicate that when the hydroxyl is *cis* to the amino group, the complex with the crown ether in that system is considerably less stable. The effect in the CE system is more dramatic and can be explained by differences in hydrogen bonding between the hydroxyl group and carboxyl groups on the crown ether. Compounds **12** and **15** are structural isomers, differing only in the orientation of the dibenzo functionality with respect to the amino and carbonyl groups. Although the structural difference in these two compounds is located far from the chiral center, there are significant differences in resolution observed using both techniques. In the HPLC system, the difference in resolution for these two compounds may be a combination of steric and hydrophobic effects. In the CE system, the effect is most likely purely steric, since the carbonyl group is in the same position relative to the amino group in both molecules.

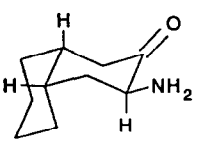
3.3. Aminodecaline analogues

The final group of compounds consists of three structural isomers (compounds **16–18**) and related compound which lacks the carbonyl moiety at the 2-position (compound **19**) (see Table 3).

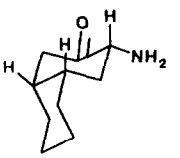
Table 3
Results for aminodecalin analogues



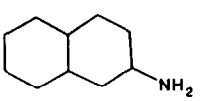
Compound **16**
(trans)



Compound **17**
(cis)



Compound **18**
(cis - 3R,9R,10S)



Compound **19**

Compound	HPLC Results				CE Results			
	t_{R1}	t_{R2}	α	R_s	t_{m1}	t_{m2}	α	R_s
16	8.71	16.50	2.06	6.15	12.31	13.07	1.062	1.45
17	7.50	9.29	1.28	2.15	12.85	14.01	1.090	3.34
18	6.03	6.27	1.05	0.42	12.89	13.55	1.051	2.26
19	28.35	–	1.00	0	12.81	–	1.000	0

Experimental conditions given in text. t_{R1}, t_{R2}, t_{m1} and t_{m2} as in Table 1.

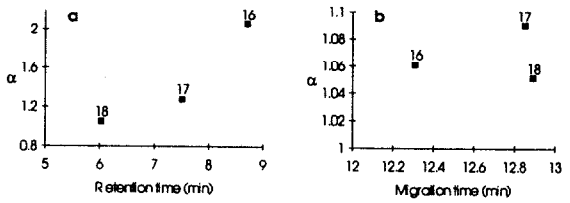


Fig. 7. (a) Correlation between separation factor, α, and HPLC retention time. (b) Correlation between α and CE migration time. Compound numbers refer to Table 3.

In the HPLC experiment, a strong correlation is observed between retention time and α (see Fig. 7a). Under the standard conditions for this series of compounds, compound **18** is only poorly resolved. By adjusting the eluent, it is possible to adequately resolve compound **18**, at the expense of analysis time. Under the eluent conditions required to resolve this compound, retention times for compounds **16** and **17** are expected to be very long. Nevertheless, it is possible to analyze all three compounds in the same run. In the CE system, all three compounds are well resolved, but no correlation between migration time and a was observed (see Fig. 7b). The retention times of all three compounds are within 0.5 min of one another. The *cis* isomers are significantly different in shape from the trans, with probably leads to the larger resolutions observed for these isomers.

A fourth compound (compound **19**) was analyzed by both HPLC and CE. This compound lacks any chromophore whatsoever. In order to detect it by HPLC, post-column derivatization with OPA was employed. Indirect UV detection was used in the CE experiment. No resolution for this compound was observed in either system. Even when the column temperature in the HPLC system was reduced, no resolution was observed. Its retention time in the HPLC system indicates that the compound interacts with the crown ether in that system, at least to some extent. Because the relationship between migration time and complexation in CE is weak, it is difficult to estimate the amount of complexation in that system. The fact that compound **19** was not resolved by either method suggests that the structures of the stereoisomers are so similar that there is no difference in the stability constants for complexes with crown ethers. Compound **19** was the only compound in the entire group of amines studied which was not resolved by either technique.

4. Conclusions

We have demonstrated the utility of crown ethers in the chiral separation of a variety of primary amines of pharmaceutical interest. For all but the most hydrophobic compounds tested, we found that within a series of related compounds, a correlation between the degree of retention on the HPLC column and chiral recognition was observed. For hydrophobic compounds this relationship was less clear, probably due to the more significant contribution of hydrophobic interactions to chromatographic retention. A correlation between CE migration time and chiral recognition was observed within some groups of compounds, but not others. This result is not surprising, since electrophoretic mobility in our system is a complex function of molecular size, shape, and charge, as well the degree of complexation with the crown ether.

In general, for those compounds having a reasonably strong chromophore in the UV, neither CE nor HPLC is clearly advantageous. In cases where both techniques give a good separation, CE is advantageous when the amount of available sample is very small. It may also be advantageous in the area of routine analysis because the high cost of a chiral HPLC column can be avoided. However, when high sensitivity is required, HPLC is advantageous because of the larger detector cell volume. Also, for a few compounds in this study, solubility problems were encountered in the totally aqueous CE electrolyte. For such compounds, HPLC may be more practical, although it may be possible to use an organic modifier in the CE buffer for these compounds. In the analysis of compounds that absorb poorly in the UV, HPLC is clearly advantageous in terms of its sensitivity.

For many of the compounds in this study, only one of the two techniques was successful in

separating the enantiomers. This is because different crown ethers are used in each of the two techniques. The two crown ethers have somewhat different mechanisms for chiral selection. Depending on their structure, some compounds are more easily separated by one or the other of the two systems. Therefore, one should regard CE and HPLC as complementary tools for enantiomeric resolution of primary amines.

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