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Enantiomeric separations of basic pharmaceutical drugs by micellar electrokinetic chromatography using a chiral surfactant, N-dodecoxycarbonylvaline

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Abstract

A novel surfactant with a chiral head group, (*R*)- or (*S*)-N-dodecoxycarbonylvaline (DDCV), was used to achieve enantiomeric separations of twenty basic pharmaceutical compounds by micellar electrokinetic chromatography (MEKC). Most of these compounds were β-agonists (anti-asthmatic, broncodilators) or β-antagonists (anti-hypertension, anti-angina). DDCV can separate polar as well as more hydrophobic chiral analytes in the same buffer system. The selectivities for these enantiomeric pairs range from 1.03 to 1.23 with good efficiencies. Separations utilizing DDCV are easy to optimize and allow for exact enantiomeric migration order reversal by switching the enantiomeric form of the surfactant. Buffer systems were assessed to minimize Joule heating and to optimize the repeatability of parameters such as migration time, relative migration time, selectivity, peak areas and area ratios. An electrolyte system consisting of 25 m*M* DDCV, 100 m*M* zwitterionic CHES (2-[N-cyclohexylamino]ethanesulfonic acid) and 10 m*M* triethylamine (TEA) was most effective for these runs. The precision for migration times, relative migration times and selectivities was better than 1%, 0.1% and 1% R.S.D., respectively, while the precision for the area ratios ranged from 1% to 4%. The possible effect of analyte structure on selectivity, efficiency and precision of peak area was studied.

Keywords: Enantiomer separation; Chiral surfactants; Micellar electrokinetic chromatography; N-Dodecoxycarbonylvaline; Chiral surfactants; Micellar electrokinetic chromatography; Acebutolol; Alprenolol; Atenolol; Benzoin; Bupivacaine; Clenbuterol; Disopyramide; Ephedrine; Isoproterenol; Metoprolol; N-Methylpseudoephedrine; Norephedrine; Norphenylephrine; Octopamine; Oxprenolol; Pindolol; Propranolol; Pseudoephedrine; Salbutamol; Synephrine

1. Introduction

A large number of pharmaceutical drugs exist in enantiomeric forms. Frequently, only one form is therapeutically active while the other is inactive or toxic. As a result it is very important to develop separation methods for these enantiomeric pairs that have almost identical physical and chemical properties. Considerable advances have been made during the last two decades in HPLC chiral separations. However, capillary electrophoresis techniques offer the advantage of high separation efficiencies, easy exchange of separation media and small volume use.

Many enantiomeric separations have been reported using chiral selectors. In free solution capillary electrophoresis (FSCE) these additives have included: cyclodextrins [1–6], carbohydrates such as maltose and dextrin [7,8], crown ethers [9,10] and ligand complexes [11,12]. Micellar electrokinetic chromatography (MEKC) has also been used as a

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chiral separation technique. MEKC methods use a surfactant such as sodium dodecyl sulfate (SDS) in combination with CDs or other natural chiral selectors like maltose or bile salts [13,14]. Optically active amino acid-derivatized surfactants, such as sodium N-dodecanoyl-L-valinate (SDVal) and N-dodecanoyl-L-serine (SDSer), have been used successfully in chiral separations of both ionic and non-ionic enantiomers [15–17]. Recently several review articles have been published on chiral separations [18–20].

Although all of these methods for enantiomeric separations have been successful, they are not universal in their applicability. In most cases, careful optimization of experimental conditions are required to achieve even a partial separation of enantiomers. Of all the chiral additives listed above, cyclodextrins have proven to be the most popular to date. However there are several disadvantages in optimizing separations with CDs [21,22]. Wren and Rowe [23–26] reported that no optimum CD concentration will be ideal for all separations and that the optimum concentration will vary depending on the nature of the analyte's affinity for the chiral selector. Native CDs have limited solubility, especially β-CD, and consequently the optimum concentration for a separation may not be attainable. Derivatized forms of CDs are generally more soluble, and there are a large number of derivatized CDs from which to choose, but the appropriate CD choice is not always evident and many of the derivatized CDs are very costly. The average degree of substitution of a derivatized CD can vary from one manufacturer to another. Commercial manufacturers of CDs do not often declare the purity, degree of substitution, and the exact position of the substitution(s) [21,22]. These purity factors can have an effect on the selectivities obtained with a particular method and, more importantly, on the reproducibility and reliability of a method. Another disadvantage of all CDs is that they only exist in one enantiomeric form. It is advantageous for quantitative determination of an enantiomeric impurity that it be eluted first in most cases. If enantiomers are not baseline resolved, errors can occur in the integration of the smaller peak, especially if the smaller peak is located on the tail of the larger peak [27]. Charged CDs have been used to selectively elute enantiomers, however the manipulation of pH parameters to achieve this is difficult [28].

A promising approach to chiral separations that overcomes most of the disadvantages of CDs is the utilization of synthetic chiral surfactants in MEKC. Dobashi (1989) and Terabe (1991–94) have had success using amino acid derivatized surfactants [15–17]. One of the commercially available synthetic chiral surfactants of interest is N-dodecoxycarbonylvaline (DDCV). This surfactant is comprised of a C_{12} hydrophobic chain with an amino acid, valine, as a hydrophilic head group. The structure of DDCV is similar to N-dodecanoylvaline, the chiral surfactant investigated by Otsuka et al. [15], but the amide group is replaced by a carbamate group (see Fig. 1). The selectivity of DDCV was initially investigated by Mazzeo et al. [29] and compared to SDVal. DDCV showed a higher selectivity for 10 out of 12 basic pharmaceutical analytes. Another result of this structural change was a lower UV absorbance at 214 nm. This allows higher concentrations of DDCV to be used without a loss of sensitivity due to the absorbance of the surfactant. DDCV has a reported solubility of up to 200 m*M* in buffer solutions above pH 7. The limiting factor for the amount of surfactant used is the conductivity of the solution due to the presence of charged anionic micelles and the buffer ions. Lower conducting organic or Goods buffers, such as HEPES, Tris or CHES, allow higher concentrations of surfactant to be used. Another essential characteristic of an effective chiral selector is a chiral center with polar groups in close proximity. With DDCV, the chiral center is the α-carbon of the amino acid valine and the polar groups are the carbamate linkage and the carboxylic acid moiety. These groups will be part of the hydrophilic layer of the micelle and will interact

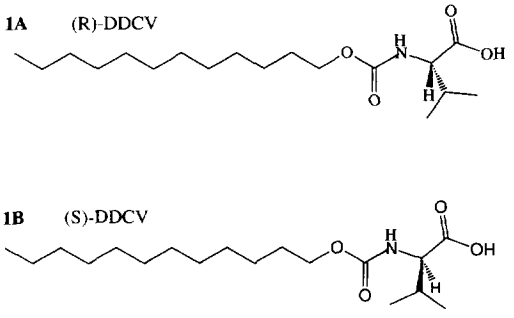

Fig. 1. Chemical structures of the chiral surfactants (*R*)- and (*S*)-N-dodecoxycarbonylvaline.

with the chiral analyte to give a stereoselective separation. The isopropyl group is important because it imparts a steric interaction which can modulate the degree of analyte interaction with the polar groups around the chiral center. A fourth important feature of DDCV is its availability in either optically pure form (*R* or *S*), making reversal of elution order as easy as switching enantiomeric forms of the surfactant. This is important not only for determining an enantiomeric impurity but also to show that the separation is an enantiomeric one.

In this study (*R*)-or (*S*)-DDCV systems were studied. Four different buffer systems were used: sodium borate, sodium phosphate and the zwitterionic buffers HEPES and CHES. Triethylamine (TEA) was used as a buffer additive to study its effect on repeatability of migration time and peak shape. Selectivities were determined as well as the repeatabilities of peak areas for the enantiomeric pairs. The compounds studied were all basic pharmaceutical amines (except benzoin, a neutral analyte): (1); acebutolol, (2); alprenolol, (3); atenolol, (4); benzoin, (5); bupivacaine, (6); clenbuterol, (7); disopyramide, (8); ephedrine, (9); isoproterenol, (10); metoprolol, (11); N-methylpseudoephedrine, (12); norephedrine, (13); norphenylephrine, (14); octopamine, (15); oxprenolol, (16); pindolol, (17); propranolol, (18); pseudoephedrine, (19); salbutamol and (20); synephrine (see Fig. 2).

2. Experimental

2.1. Instrumental

A Waters Quanta 4000 or 4000E capillary electrophoresis system (Waters, Milford, MA, USA) equipped with fixed-wavelength UV detection at 214 nm was employed for all the separations performed in this study. All of the test analytes were detectable at this wavelength. MEKC was performed in a 75 μm I.D. (363 μm O.D.) fused-silica capillary tube ranging in length from 52 to 55 cm (45 to 48 cm from inlet to detector) (Polymicro Technologies, Phoenix, AZ, USA). Injections were made hydrostatically for 2 s. The applied voltage for the studies was 12 kV, unless otherwise noted in the text. The data from the Quanta 4000 were collected and processed on a Macintosh IIci (Cupertino, CA, USA) using a MacLab 4 channel ADC with the appropriate software (AD Instruments, Milford, MA, USA). The data from the Quanta 4000E were collected and processed on a NEC Image 466es (Milford, MA, USA) using Millennium 2000 or 2010 software (Waters, Inc. Milford, MA, USA). All experiments were done at ambient temperature (25°C)

2.2. Materials

The zwitterionic buffers, CHES (2-[N-cyclohexylamine]ethanesulfonic acid), and HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) were purchased from Sigma (St. Louis, MO, USA) and Aldrich (Milwaukee, WI, USA), respectively. The chiral surfactants, (*R*) and (*S*)-dodecoxycarbonylvaline (Waters EnantioSelect™ (*R*)-and (*S*)-Val-1), were provided by Waters (Milford, MA, USA). Triethylamine was purchased from J.T. Baker (Phillipsburg, NJ, USA). The following racemic or pure enantiomeric forms of chiral analytes were purchased from Sigma (St. Louis, MO, USA): racemic atenolol, (1*R*,2*R*)-(−)-N-methylpseudoephedrine, racemic bupivacaine, racemic metoprolol, racemic disopyramide, racemic octopamine, racemic clenbuterol, racemic acebutolol, racemic alprenolol, (*S*)-(−)-alprenolol, racemic isoproterenol, (*S*)-(+)-isoproterenol, racemic oxprenolol, (1*S*,2*S*)-pseudoephedrine, (1*R*,2*R*)-pseudoephedrine, racemic salbutamol and racemic synephrine. The following racemic or pure enantiomeric forms of chiral analytes were purchased from Aldrich (Milwaukee, WI, USA): (*R*)-(+)-atenolol, (*S*)-(−)-atenolol, (1*S*,2*S*)-(+)-N-methylpseudoephedrine, racemic pindolol, racemic norphenylephrine, racemic ephedrine, (1*S*,2*R*)-(+)-ephedrine, racemic norephedrine and (1*S*,2*R*)-(+)-norephedrine.

All repeatability studies were performed in MEKC buffer solutions containing 25 m*M* DDCV and 10 m*M* TEA. The sodium borate and sodium phosphate concentrations were 25 m*M*. The concentrations of CHES and HEPES were both 100 m*M*. MEKC electrolyte solutions were prepared by weighing out the surfactant and the appropriate buffers and adding HPLC grade distilled water (J.T. Baker, Phillipsburg, NJ, USA). The solutions were sonicated in a beaker and the pH monitored and adjusted with 1.0 *M*

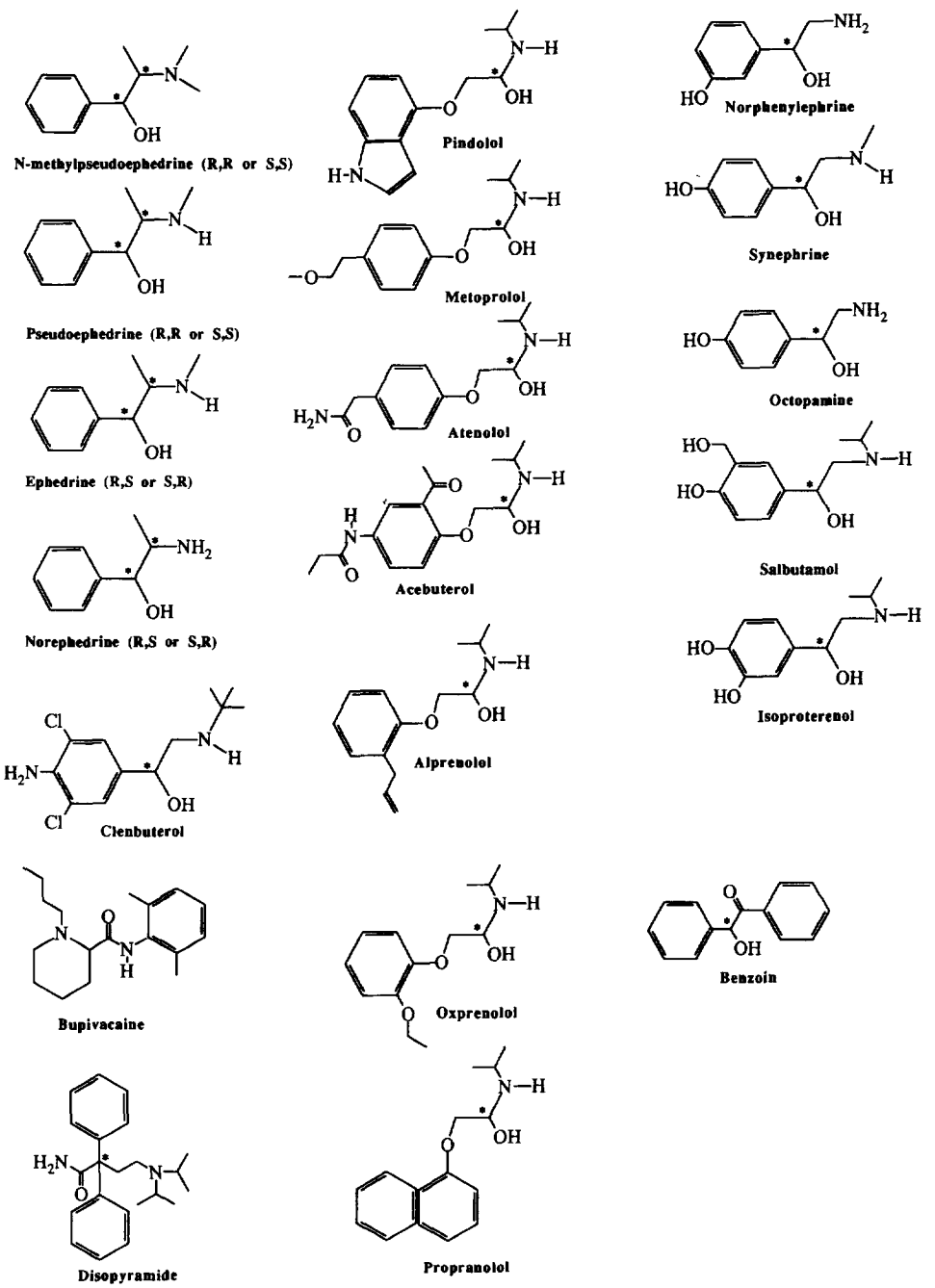

Fig. 2. Chemical structures of the analytes.

sodium hydroxide (NaOH) to keep the pH above 7. The contents of the beaker were transferred to an appropriate volumetric flask and enough TEA was added to make a final concentration of 10 m*M*. The final pH was adjusted to 8.8 with 1.0 *M* sodium hydroxide, unless otherwise noted in the text. The CZE buffer solutions for measurement of $t_{OS,CZE}$ (see equation 1 section 2.4) were made in the same manner but with the DDCV omitted. All of these experiments were conducted using a Waters 4000

with a MacLab data acquisition system and a Peaks 1.3 software for the integrations. The baseline drawn by Peaks program does not provide the same consistency as the Millenium 2000 integration software used on the Waters Quanta 4000E system.

Stock sample solutions of the chiral analytes were prepared in methanol at concentrations of 2.00 to 4.00 mg/ml and diluted with running buffer solution to a final concentration of 0.10 to 0.25 mg/ml (≤5% methanol). The final dilution also contained a t_{mc} marker, either decanophenone or sulcanazole, at a concentration of ~0.06 mg/ml.

2.3. Methods

The capillary was activated by first purging with 1.0 *M* NaOH for 20 min followed by a purge of 0.1 *M* NaOH for 20 min. The capillary was then rinsed and equilibrated with the running buffer. The equilibration time was 3 h at the running voltage as described by Cohen and Grushka [30]. The capillary was used only with buffer solutions containing 10 m*M* TEA to maintain the conditioning of the capillary wall and enhance the repeatability of separations.

Electroosmotic velocities were measured using a method previously published [31]. For each separation the t_{mc} values, which represent the elution time of the DDCV micelle, were measured using decanophenone or sulcanazole. These values were confirmed with the iterative computation method developed by Bushey and Jorgenson [32]. Equations used in the calculations of retention factors, selectivities, and efficiencies are discussed in Section 2.4.

To examine the repeatability of migration time and peak areas, separations were performed in sets of nine. Within each set of experiments, the capillaries were purged for 3 min with running buffer. Between each set of experiments, the capillary was purged with 0.1 *M* NaOH for 5 min. The CZE separations were done in sets of three. Since we were using charged analytes, it was necessary to determine their electrophoretic mobility in the absence of micelles to calculate their retention factors. Previous experiments performed below the critical micelle concentration (0.5 m*M* DDCV) indicated that ion pairing was minimal [29] and therefore the cationic analytes have an apparent mobility equal to the sum of the electroosmotic mobility and electrophoretic mobility.

2.4. Calculations

The electrophoretic migration times of charged analytes not interacting with the micelle, t_{aq}, were calculated using Eq. (1):

$$t_{aq} = \frac{1}{\left(\frac{1}{t_{CZE}} + \frac{1}{t_{OS.MEKC}} - \frac{1}{t_{OS.CZE}}\right)} \quad (1)$$

where t_{CZE}, $t_{OS.MEKC}$, and $t_{OS.CZE}$ are the migration times of the charged analytes in free solution, the electroosmotic flow marker (methanol) in the presence of DDCV micelles and the electroosmotic flow marker in FSCE [33].

The retention factors, k, for the two enantiomers were determined using Eq. (2):

$$k = \frac{(t_R - t_{aq})}{t_{aq}\left(1 - \frac{t_R}{t_{mc}}\right)} \quad (2)$$

where t_R and t_{mc} are the migration time of the analyte and micelle, respectively. The method for t_{mc} measurement is described in section 2.3.

The selectivities, α, were determined using Eq. (3):

$$\alpha = \frac{k_2}{k_1} \quad (3)$$

where k_1 and k_2 were the retention factors for the 1st and 2nd enantiomer, respectively.

Efficiencies, N, were calculated using the Foley–Dorsey Eq. (4):

$$N_{SYS} = \frac{41.7(t_R/W_{0.1})^2}{B/A + 1.25} \quad (4)$$

where t_R is the retention time of the enantiomer, $W_{0.1}$ is the width at 10% of the height, and B/A is the asymmetry factor.

The resolutions for the charged solutes were calculated using Eq. (5) derived by Foley and Nielsen.

$$R_s = \left(\frac{\sqrt{N}}{4}\right)\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2}{1 + k_{avg}}\right) \times \left(\frac{1 + \mu_r - (t_o/t_{mc})}{1 + \mu_r + (t_o/t_{mc})k_{avg}}\right) \quad (5)$$

where N is the average efficiency for the enantiomeric pair and μ_r is t_o/t_{ep}. The parameter t_o is the migration time of a neutral analyte that does not interact with the micelles, and t_{ep} is the time it would take an analyte to migrate the length of the column in the absence of micelles and electroosmotic flow [34]. The latter is not equivalent to any of the parameters in Eqs. (1) or (2).

3. Results and discussion

3.1. Selection of buffer system

Inorganic buffers, such as sodium phosphate–borate, are often used in CE and MEKC. High concentrations of these buffers have been used to decrease the adsorption of basic amines to the capillary wall. This adsorption most readily occurs at higher pH where more silanol groups are ionized. The primary drawback associated with the use of inorganic buffers is excessive operating currents and the resultant Joule heating effects. These effects can cause temperature gradients within the capillary, leading to band broadening and loss of resolution. As a result, low voltages must be employed.

In the initial study using DDCV, a mixture of 25 m*M* sodium borate and 25 m*M* phosphate was used. This buffer system's power/length was above 2.0 W/m for applied voltages as low as 12 kV. Several other buffer systems were investigated. All buffer systems in the study contained 25 m*M* DDCV. In addition to the inorganic buffers, zwitterionic buffers were investigated because they offer lower conductivity when the buffer is adjusted close to its pK_a. Therefore higher concentrations can be used with minimal contributions to the operating current and the resultant Joule heating. Higher buffer concentrations can also reduce interactions between the basic solutes and the capillary walls resulting in better peak shape and repeatability [35]. This is especially critical in enantiomeric separations where the detection of small amounts of the minor enantiomer is necessary. However, zwitterionic buffers will give shorter migration windows in comparison to inorganic buffers of comparable concentrations. Zwitterionic buffers have generally a faster electroosmotic flow (EOF). The Ohm's law plots for all the experimental buffer systems are shown in Fig. 3.

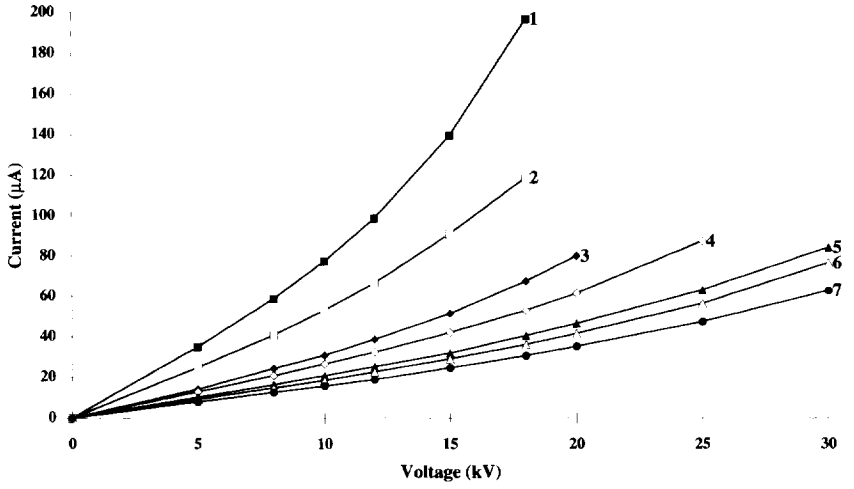

Fig. 3. Ohm's law plots for all of the buffer systems studied. All buffer systems contain 25 m*M* (*R*)-DDCV surfactant, pH 8.8. (1); 25 m*M* borate/25 m*M* phosphate, (2); 25 m*M* phosphate, (3); 25 m*M* borate, (4); 15 m*M* borate/50 m*M* CHES, (5); 100 m*M* CHES/10 m*M* TEA, (6); 50 m*M* HEPES/10 m*M* TEA, (7); 50 m*M* CHES/10 m*M* TEA.

Where the plots deviate from linearity, the effects of Joule heating may have an effect on peak shape and resolution [36].

The use of triethylamine (TEA) was also studied. Cohen and Grushka [30] have reported that TEA is effective in improving peak shapes and migration time repeatability. TEA diminishes changes in the electroosmotic flow (EOF) by competing with or preventing basic solutes from adsorbing to the silica wall. The effect of TEA concentration present in the buffer system was evaluated in terms of repeatability of migration time, peak area and area ratios for the racemic drug, ephedrine. The R.S.D.s for nine consecutive runs are shown in Table 1. The buffer systems were adjusted to pH 8.8 after the addition of the TEA to 25 m*M* DDCV and 100 m*M* CHES. The results indicate a definite improvement in R.S.D.s for migration time and peak area, but there appears to be a leveling off above 10 m*M* TEA.

3.2. Enantioselectivity

The best MEKC running electrolyte that offered not only low conductivity but also good resolution was 25 m*M* DDCV, 100 m*M* CHES and 10 m*M* TEA. The power/length was below 0.5 W/m for a capillary length of 55 cm using a running voltage of 12 kV. Most of the analytes were separated in less than 20 min. Electropherograms for the analytes using this buffer system are shown in Fig. 4, Fig. 5 and Fig. 6. Note that individual optical isomers were labeled only if they were available in pure enantiomeric form so that the migration order could be determined. The neutral analyte benzoin required a concentration of 50 m*M* DDCV (see Fig. 7). Some of the more hydrophobic analytes required the addition of an organic modifier, acetonitrile, to decrease partitioning and improve resolution (see Fig. 8).

Table 2 shows the enantioselectivity for each of the analytes tested under optimum conditions. Disopyramide and acebutolol showed the lowest enantioselectivity and N-methylpseudoephedrine the highest. The solutes can be grouped according to similarities in their structure. Almost all of the analytes are amino alcohols, where the amine and hydroxy functional groups surround the chiral center(s). The first major group consists of norephedrine, ephedrine, pseudoephedrine and N-methylpseudoephedrine. They have two chiral centers and vary in their degree of amine methylation. Enantioselectivity is clearly correlated with absolute configuration and degree of methyl substitution. For example the pseudoephedrines have absolute configurations comprised of (*R*,*R*) or (*S*,*S*), whereas ephedrine and norephedrine are (*R*,*S*) and (*S*,*R*) respectively. The unusually high selectivity appears to result from a steric effect of the methyl groups. Norephedrine, with no methylation, has a selectivity of 1.06 while N-methylpseudoephedrine, a tertiary amine, has the largest value, 1.28. Pure enantiomeric form(s) of these solutes were available for analysis. The first and second chiral centers are the carbons with the alcohol and amine functionalities, respectively. The electropherograms showed in all cases that the enantiomer with *R* as the first chiral center eluted last when using *R*-DDCV. This order was reversed if *S*-DDCV was used. There was also a greater enantioselectivity if both chiral centers had the same absolute configurations.

It is interesting to note that all 4 stereoisomers of ephedrine and pseudoephedrine (Fig. 2), can be baseline resolved in a single run (see Fig. 9). To the best of our knowledge, such resolution has not yet been achieved by chiral HPLC and has only been reported one other time in electrokinetic chromatography, where a sulfobutyl ether-derivatized β-CD

Table 1
Effect of triethylamine (TEA) on chromatographic precision[a,b]

[TEA] Buffer (m*M*)	t_{R1}	t_{R2}	Area peak 1	Area peak 2	Area ratio
0	1.0	1.0	5.3	5.6	4.6
5	0.30	0.30	3.7	4.3	2.5
10	0.11	0.11	2.1	1.8	2.9
20	0.15	0.20	1.4	2.8	2.3

[a] Percent relative standard deviation.
[b] Analyte is ephedrine in 25 m*M* DDCV, 100 m*M* CHES, pH 8.8.

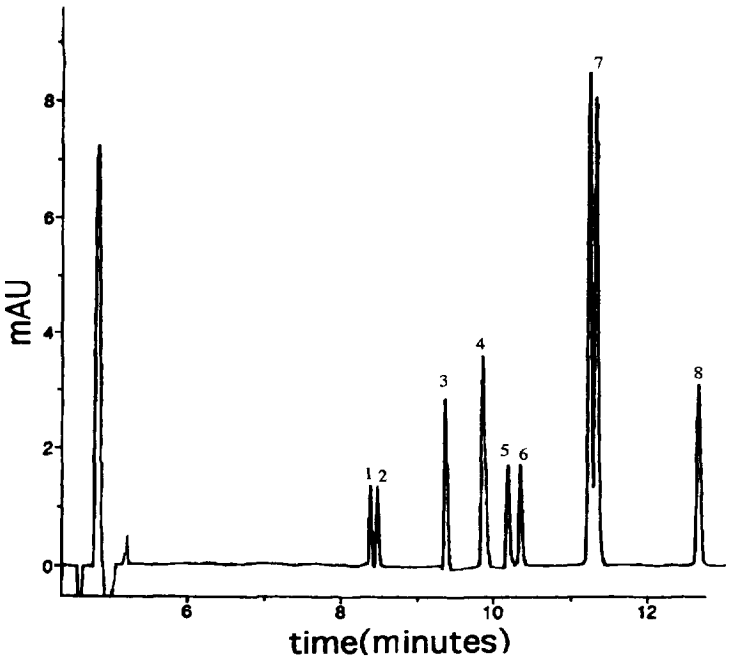

Fig. 4. Chiral MEKC separation of racemic solutes with 25 m*M* (*R*)-DDCV, 100 m*M* CHES and 10 m*M* TEA, pH 8.8. Applied voltage 12 kV, current ≤ 30 μA, power ≤ 0.6 W/m. Peaks: 1 = (*R*)-atenolol, 2 = (*S*)-atenolol, 3 = (*S,S*)-N-methylpseudoephedrine, 4 = (*R,R*)-N-methylpseudoephedrine, 5 = (1*S*,2*R*)-ephedrine, 6 = (1*R*,2*S*) ephedrine, 7 = racemic pindolol, 8 = sulcanazole (t_{mc}).

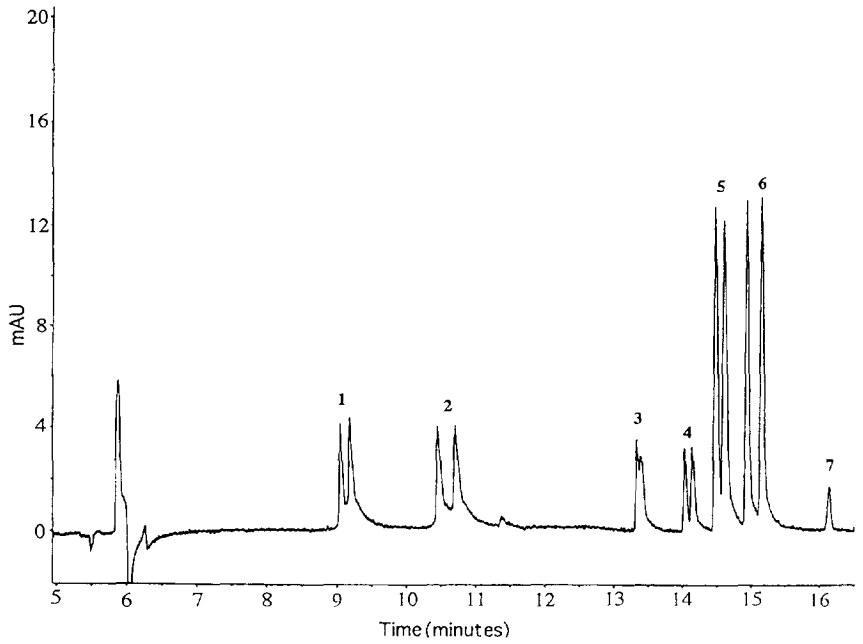

Fig. 5. Chiral MEKC separation. Peaks: 1 = octopamine, 2 = norphenylephrine, 3 = disopyramide, 4 = metoprolol, 5 = bupivacaine, 6 = clenbuterol, 7 = decanophenone (t_{mc}). Conditions as in Fig. 4.

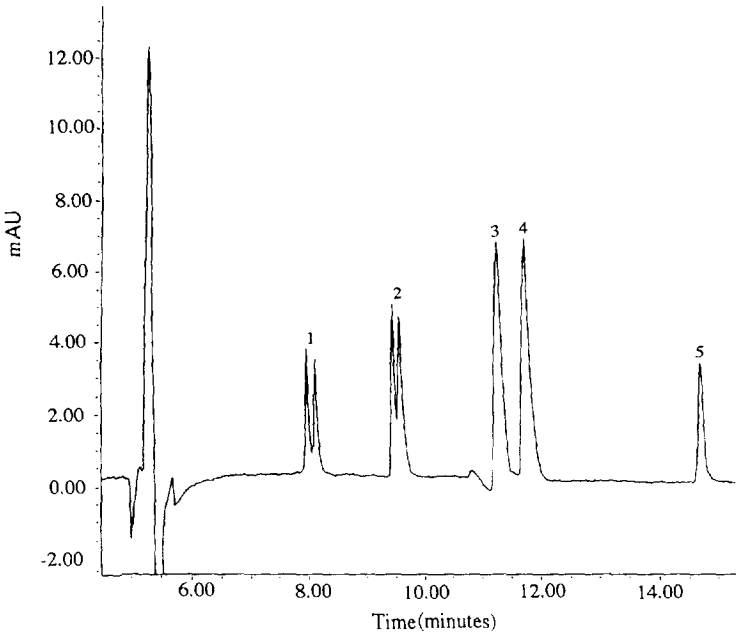

Fig. 6. Chiral MEKC separation. Peaks: 1 = racemic synephrine, 2 = racemic salbutamol, 3 = (*S,S*)-pseudoephedrine, 4 = (*R,R*)-pseudoephedrine, 5 = decanophenone (t_{mc}). Conditions as in Fig. 4.

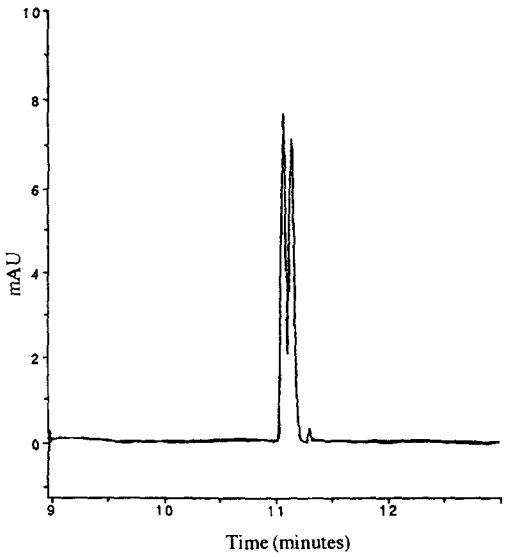

Fig. 7. Separation of racemic benzoin using a separation buffer of 50 m*M* (*R*)-DDCV. Other conditions as in Fig. 4.

was employed as the pseudostationary phase and the elution order was precisely the same [37]. In contrast to the cyclodextrin-based separation, however, a simple reversal of the elution order was easily achieved by switching from the *S*-form to the *R*-form of DDCV, keeping all other conditions constant (pH, buffer composition, organic solvent, etc.). This is not possible with cyclodextrin-based separations, which nearly always require significant changes in experimental conditions.

The second major group is comprised of acebutolol, alprenolol, atenolol, metoprolol, oxprenolol, pindolol and propranolol. These solutes contain an amino alcohol side chain bonded to the aromatic system with an ether linkage. The amine group in this case has an isopropyl substitution. The aromatic systems vary in the type of substitution which causes these analytes to have a large range of hydrophobicity. The more hydrophobic solutes such as pindolol and metoprolol elute closer to the t_{mc} marker, while atenolol with a polar amide group *para* to the amine alcohol side chain elutes relatively early. The other solutes, oxprenolol, alprenolol, propranolol and acebuterol, either elute with the t_{mc}

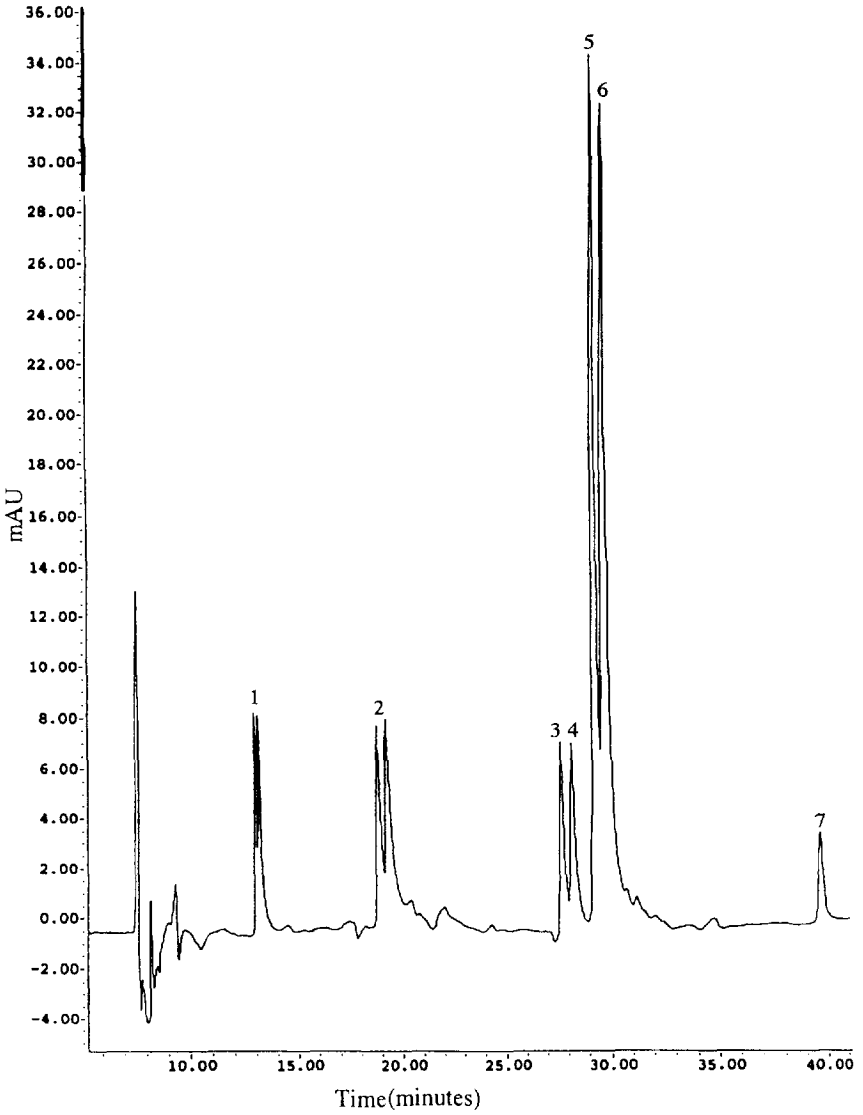

Fig. 8. Separation of racemic solutes with 25 m*M* (*R*)-DDCV, 25 m*M* sodium borate, 10 m*M* TEA and 25%(v/v) ACN, pH 8.8. Applied voltage 12 kV, current≤40 μA, power≤1 W/m. Peaks: 1 = racemic acebuterol, 2 = racemic oxprenolol, 3 = (*R*)-alprenolol, 4 = (*S*)-alprenolol, 5 = (*R*)-propranolol, 6 = (*S*)-propranolol, 7 = decanophenone (t_{mc}).

propranolol and acebuterol, either elute with the t_{mc} marker or penetrate too deeply in the micelle to interact with the chiral center of DDCV. However, they are enantiomerically separated with the use of 25% acetonitrile (ACN). Enantiomeric pure form(s) of propranolol, atenolol and alprenolol were available. In this case, when racemic mixtures were spiked, the electropherograms showed that the *S*-isomer eluted later with the *R*-DDCV. Selectivities for this group of compounds varied from 1.03 to 1.08. The more hydrophobic analytes had the higher selectivities.

The third group of amino alcohols are comprised of norphenylephrine, octopamine, synephrine, salbutamol and isoproterenol; these compounds have phenolic substitutions on the benzene ring. Again the amine groups differ in the degree of methylation for these analytes. Norphenylephrine and octopamine

Table 2
Enantioselectivities of the analytes using DDCV[a]

Chiral analyte	Enantioselectivity
Acebutolol[b]	1.03
Alprenolol[b]	1.07
Atenolol	1.04
Benzoin[c]	1.04
Bupivacaine	1.09
Clenbuterol	1.25
Disopyramide	1.03
Ephedrine	1.10
Isoproterenol	1.05
Metoprolol	1.07
N-Methylpseudoephedrine	1.28
Norephedrine	1.06
Norphenylephrine	1.09
Octopamine	1.05
Oxprenolol[b]	1.06
Pindolol	1.08
Propranolol[b]	1.08
Pseudoephedrine	1.23
Salbutamol	1.05
Synephrine	1.07

[a] Buffer systems: 25 m*M* *R*-DDCV, 100 m*M* CHES, 10 m*M* TEA, pH 8.8, unless otherwise noted in table.
[b] 25 m*M* R-DDCV, 25 m*M* sodium borate, 25% ACN, 10 m*M* TEA, pH 8.8.
[c] 50 m*M* R-DDCV, 100 m*M* CHES, 10 m*M* TEA, pH 8.8.

have no methyl groups and therefore are free primary amines. Synephrine has one methyl and both salbutamol and isoproterenol have isopropyl groups substituted on the amine group. As a whole, these solutes are very polar because of possible ionization of the phenolic groups. These negatively charged polar groups may experience repulsion from the negatively charged micelle contributing to the significant tailing shown by these solutes. Selectivities for this group of compounds range from 1.05 to 1.09.

3.3. Repeatability studies of selected analytes using a R-DDCV buffer system

Repeatability studies were performed using the buffer system of 25 m*M* DDCV, 100 m*M* CHES, and 10 m*M* TEA. The precision was calculated with respect to migration time, relative migration time and selectivity (see Table 3). The R.S.D.s of both relative migration time and selectivity reflect the consistency of DDCV at distinguishing between these optical isomers. However, selectivity is more informative. It is not dependent on the type of CE instrument used and therefore is more universal and statistically important. All analytes showed R.S.D.s below 1% for migration time. Relative migration times and selectivities had R.S.D.s of less than 0.1% and 1%, respectively. These values are comparable to those reported in the literature for enantiomeric separations using CDs in CE [38–40].

A group of analytes ranging from hydrophilic to hydrophobic were selected for a repeatability study on area determination and efficiencies. The enantioselectivity of the solutes chosen had to be large enough to give sufficient resolution for area determination. The selected enantiomers were representatives from the three groups cited earlier in section 3.2. The precision for peak area and area ratios are shown in Table 4 for these nine test solutes. Table 5 gives the resolution, asymmetry factors and efficiencies for the enantiomers. The R.S.D.s for peak area and area ratio range from 1.5–5.8% and 1.3–4.4% respectively.

The β-blockers (atenolol, pindolol and metoprolol) have better peak shape and therefore more consistent peak area determinations. The asymmetry factor (B/A) for these compounds ranged from 1.81 to 2.29 with the more hydrophobic analyte, pindolol, having the lowest asymmetry factor. The R.S.D.s for peak area ratio ranged from 1.5 to 3.6. The solutes with an ether linkage are generally hydrophobic and elute later. They spend more time in the micelle and therefore have less exposure to the capillary wall. The solutes ephedrine and N-methylpseudoephedrine have R.S.D.s for peak area ratios of 3.7 and 1.3 respectively. The ephedrine isomers have better peak shapes with asymmetry factors of 2.41 and 1.97, respectively, than N-methylpseudoephedrine with asymmetry factors of 4.97 and 4.19. The R.S.D. of peak area ratio for ephedrine, however, is greater than that of N-methylpseudoephedrine. The N-methylpseudoephedrine isomers are baseline resolved allowing the peak areas to be more accurately assessed. The hydroxyphenylamines (norphenylephrine and octopamine) are very polar and have free aliphatic primary amines. They have the highest R.S.D. values for peak area ratio. Their asymmetry factors range from 3.08 to 3.77 and show considerable tailing. These particular analytes, more so than the others, spent less time in the micelle and

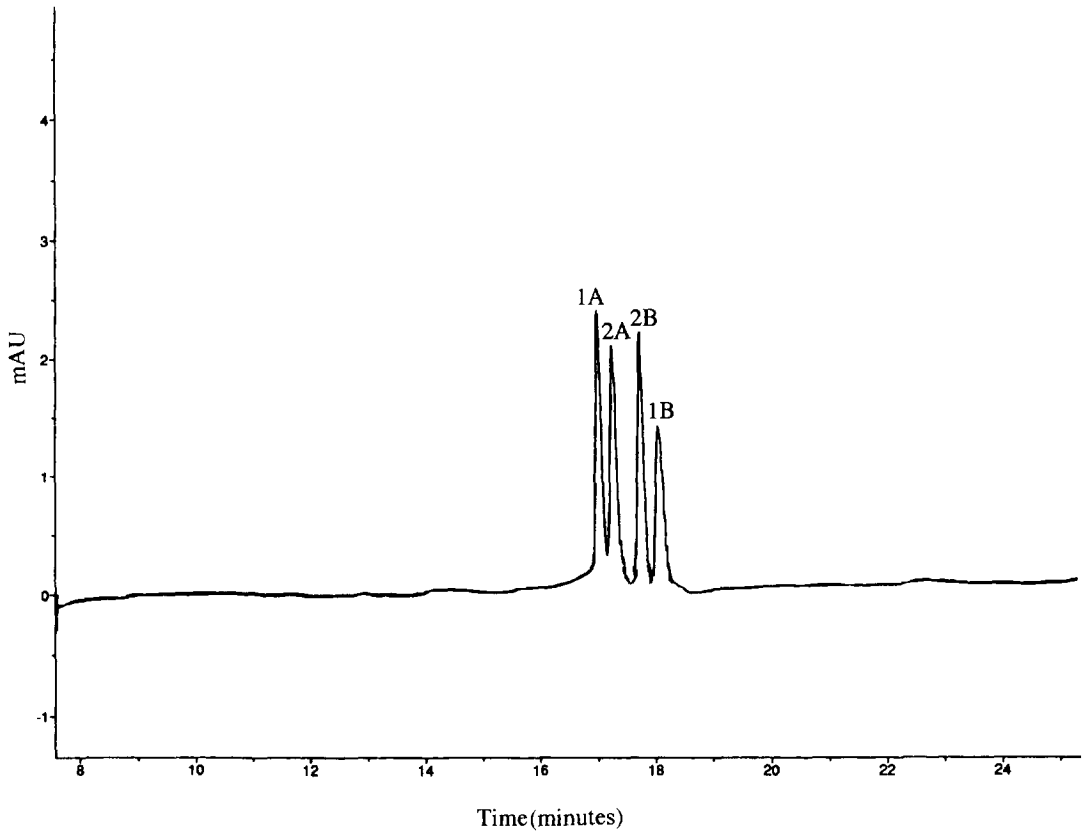

Fig. 9. Separation of stereoisomers of pseudoephedrine and ephedrine with 25 m*M* (*S*)-DDCV. Peaks: 1A = (*R*,*R*)-pseudoephedrine, 1B = (*S*,*S*)-pseudoephedrine, 2A = (*R*,*S*)-ephedrine, 2B = (*S*,*R*)-ephedrine. Other conditions as in Fig. 4.

Table 3
R.S.D.'s for migration time, relative migration and selectivity

Analyte	R.S.D. (%)			
	t_{R1}	t_{R2}	Relative migration (t_{R2}/t_{R1})	Selectivity
Atenolol	0.40	0.30	0.10	0.50
Bupivacaine	0.48	0.46	0.05	0.46
Clenbuterol	0.47	0.45	0.07	1.00
Disopyramide	0.54	0.55	0.10	0.39
Ephedrine	0.26	0.29	0.05	0.45
Isoproterenol	0.23	0.27	0.07	0.28
Metoprolol	0.52	0.51	0.05	0.37
N-Methylpseudoephedrine	0.26	0.28	0.04	0.78
Norphenylephrine	1.00	0.99	0.01	0.18
Octopamine	1.00	1.10	0.10	0.38
Pindolol	0.33	0.44	0.10	1.00
Pseudoephedrine	0.81	0.86	0.08	0.30
Salbutamol	0.53	0.52	0.01	0.10
Synephrine	0.37	0.42	0.20	0.80

Table 4
Precision for absolute areas and area ratios

Analyte	R.S.D. (%)		
	$Area_1$	$Area_2$	Area ratio
Atenolol	2.1	2.0	1.7
Bupivacaine	2.0	2.2	2.0
Clenbuterol	2.0	1.9	1.6
Ephedrine	3.6	4.1	3.7
Metoprolol	4.7	5.8	3.6
N-Methylpseudoephedrine	2.5	2.3	1.3
Norphenylephrine	3.4	4.2	4.4
Octopamine	3.7	3.5	3.8
Pindolol	1.4	2.6	1.5

Buffer is 25 m*M* DDCV, 100 m*M* CHES, 10 m*M* TEA, pH 8.8.

therefore have the most exposure to the capillary wall.

The efficiencies for the later eluting solute peaks are considerably higher than those for the earlier polar solute peaks. The second enantiomer to elute of a racemic mix had a lower efficiency. This is expected if the second peak is on the tailing edge of the first enantiomer. N-Methylpseudoephedrine has the largest selectivity and the best resolution. The efficiencies for these peaks are very similar and do not have the large differences shown for other pairs of enantiomers.

4. Conclusions

The results of this investigation show that the chiral surfactant DDCV is very effective in achieving good enantiomeric separations for a large number of basic pharmaceutical compounds. The enantiomers of acebuterol could not be separated by the use of several different CD derivatives but showed enantioselectivity with DDCV [3,41]. A group of analytes with a large range of hydrophobicity have been separated using the same low concentration, 25 m*M*, of either *R* or *S*-DDCV. This offers an advantage over other chiral selectors where the optimum—concentration can vary greatly from solute to solute.

The unique buffer system used offers several advantages. First the zwitterionic buffer, CHES, allows for large increases in the ionic strength without large increases in the current during the separation. This avoids the potential for band broadening due to excessive Joule heating. Secondly the addition of TEA stabilizes changes in EOF by preventing either solute or surfactant interactions with the capillary wall. The use of TEA lessens the need to rinse with NaOH between runs. Frequent rinsing with NaOH can cause changes in the chemistry of the capillary wall. The repeatabilities in migration times and selectivities were excellent with R.S.D.s of less than 1%. A precision of less than 1% R.S.D. will allow qualitative identification of components in a mixture.

The R.S.D.s of peak area ratios for the enantiomers in this study indicate the importance of selectivity and peak shape for quantitative analysis. Clenbuterol, which has a selectivity of 1.25 and enantiomeric peaks with asymmetry factors of 1.9, has the best R.S.D., 1.6. DDCV gave good selec-

Table 5
Resolution, capacity factors, asymmetries and efficiencies for test solutes[a,b]

Analyte	Resolution	First enantiomer			Second enantiomer		
		k	*B/A*	*N*	*k*	*B/A*	*N*
Atenolol	1.7	2.79	2.26	187 000	2.92	1.87	150 000
Bupivacaine	1.5	15.7	2.64	173 000	17.1	2.58	100 000
Clenbuterol	3.3	28.9	1.92	235 000	36.0	1.94	202 000
Ephedrine	3.0	7.73	2.41	181 000	8.49	1.97	123 000
Metoprolol	1.6	14.9	2.29	226 000	15.9	2.10	180 000
N-Methylpseudoephedrine	4.5	5.04	4.97	34 000	6.42	4.19	48 000
Norphenylephrine	1.8	10.4	3.77	46 000	10.6	3.08	44 000
Octopamine	1.3	2.06	3.43	80 000	2.17	2.97	58 000
Pindolol	1.5	13.8	1.85	185 000	14.8	1.81	117 000

[a] Asymmetries measured at 10%, efficiencies by Foley–Dorsey equation [33].
[b] Same conditions as in Table 4.

tivities and peak shapes for the beta-blockers, atenolol, metoprolol and pindolol, in the repeatability study. The R.S.D.s for peak area ratio for these compounds were close to regulatory standards. Other beta-blockers separated by DDCV, but not in the repeatability study, also have good selectivity and peak shape. The other alkanolamine analytes have good selectivities and reasonable R.S.D.s even though tailing is evident. Further modifications of the buffer system may lead to improvement in the R.S.D.s for quantitative purposes.

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