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Chiral separation of basic drugs using cyclodextrins as chiral pseudo-stationary phases in capillary electrophoresis

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ABSTRACT

Capillary electrophoresis was used for the chiral resolution of basic racemic drugs in general and in particular for dimethindene and four possible metabolites. Conditions for optimum enantioselectivity and resolution were determined by changing the cyclodextrin type, cyclodextrin concentration, pH of the run buffer, applied current and capillary temperature.

INTRODUCTION

Enantiomer resolution is an important field in analytical chemistry, especially in pharmaceutical analysis. About 88% of synthetic chiral drugs are sold as racemates [1], and often one of the enantiomers is more active than the other or is responsible for inadvertent side-effects. For the investigation of the metabolism of racemic therapeutic agents, GC [2] and HPLC [3] are widely used. During the last few years, capillary electrophoresis (CE) [4] has developed into a powerful tool, including applications in chiral analysis [5]. Enantiomeric separations can be achieved using chiral buffer additives such as cyclodextrins (CD) [6,7].

The metabolism of dimethindene maleate, N,N - dimethyl - 3 - [1-(2-pyridinyl)ethyl] -1H-indene-2-ethanamine maleate, has been studied by Radler and Blaschke [8] and Prien and Blaschke [9] using protein- and cellulose-based chiral stationary phases for enantiomeric separations by HPLC.

In this work we used CE with run buffers containing different cyclodextrins to resolve dimethindene and four possible metabolites into their enantiomers in one run. A number of parameters influencing the selectivity and resolution of the compounds were investigated. Further, a number of structurally different racemic basic drugs were resolved by CD-containing run buffers.

EXPERIMENTAL

Chemicals and reagents

Dimethindene maleate was obtained from Zyma (Nyon, Switzerland). N-Demethyldimethindene, N-demethyl-6-methoxydimethindene, 6-methoxydimethindene and the enantiomers of dimethindene [(*S*)- (+)- and (*R*)- (−)-] were prepared by Radler and Blaschke [8] and dimethindene-N-oxide was synthesized by Prien and Blaschke [9]. α-, β- and γ-cyclodextrin (α-, β- and γ-CD), methyl-β-cyclodextrin [ME-β-CD, molar substitution $(MS) = 1.8$], hydroxyethyl-β-cyclodextrin (HE-β-CD, $MS = 1.0$), hydroxypropyl-β-cyclodextrin (HP-β-CD, $MS = 0.9$) and hydroxypropyl-β-cyclodextrin (HP-β-

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CD, $MS = 0.6$) were a kind gift from Wacker-Chemie (Munich, Germany). KH_2PO_4, Na_2HPO_4, H_3PO_4, NaOH (all of analytical-reagent grade) were purchased from Merck (Darmstadt, Germany). Other racemic drugs were gifts from the manufacturers.

Apparatus for CE

A Grom (Herrenberg, Germany) capillary electrophoresis System 100, equipped with an HP 3396 A integrator (Hewlett-Packard, Avondale, PA, USA) and a Linear Instruments (Reno, NV, USA) UVIS 200 detector, and a Beckman (Munich, Germany) P/ACE 2100 capillary electrophoresis system were used with an untreated fused-silica capillary (Grom) of 40 cm effective length × 50 μm I.D.

Standard operating conditions, unless stated otherwise, were as follows: effective voltage, 400 V/cm; temperature, 21 ± 1°C; sample introduction, (a) Grom, hydrostatic 10 cm, 30 s and (b) Beckman, low pressure, 10 s; detection, (a) UV at 205 nm and (b) UV at 200 nm; anode and cathode buffers with the same pH and molarity as the run buffer, but containing no CD, were used.

Buffer and sample preparation

Stock solutions of 50 m*M* KH_2PO_4 and 50 m*M* Na_2HPO_4 buffers were prepared in doubly distilled, deionized water, filtered and degassed in an ultrasonic bath before use. The pH was adjusted, if possible, with the stock buffer solutions, otherwise with 50 m*M* H_3PO_4 or 50 m*M* NaOH. Run buffers were prepared accordingly after addition of an appropriate amount of cyclodextrins.

Stock solutions of 1 mg/ml racemic drug were prepared, stored at 4°C and diluted to 60 μg/ml before use.

RESULTS AND DISCUSSION

The endoosmotic mobility was determined using mesityl oxide as a neutral marker. Fig. 1 shows the electroosmotic mobility in the pH range 3–10. The magnitude of the endoosmotic flow (μ_{eof}) was calculated using the equation

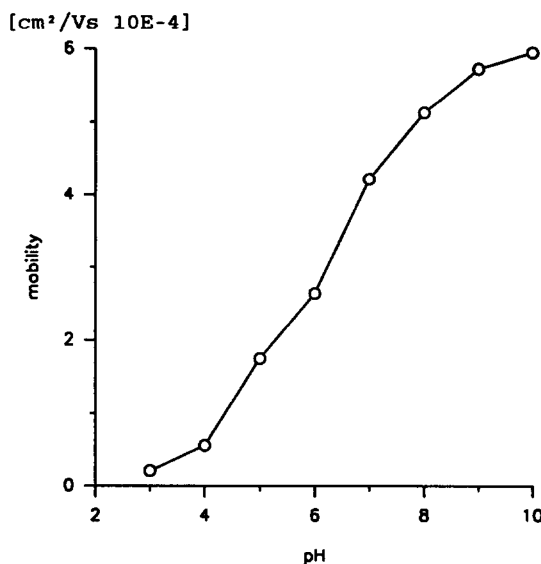


Fig. 1. Endoosmotic flow over the pH range 3–10, determined using mesityl oxide as a neutral marker. Conditions: run buffer, 50 m*M* phosphate buffer; capillary, untreated fused silica, 45 cm length to detector, 62 cm total length.

$$\mu_{eof} + \mu_{eph} = \frac{L}{tE} \quad (1)$$

where L is the capillary length (cm), t the migration time (s) and E the effective voltage (V/cm) [10]. With a neutral marker such as mesityl oxide, no electrophoretic mobility (μ_{eph}) occurs, so that the observed values are due only to the endoosmotic mobility (μ_{eof}).

The separation selectivity (α) in CE can be expressed as the ratio of the effective mobilities using the equation

$$\alpha = \frac{\mu_{eph1}}{\mu_{eph2}} = \frac{\mu_{app1} - \mu_{eof}}{\mu_{app2} - \mu_{eof}} \quad (2)$$

where the subscripts 1 and 2 refer to the earlier and later migrating species, respectively [11]. The effective mobility (μ_{eph}) is calculated as the difference between the apparent mobility (μ_{app}) and μ_{eof}. Because it was not possible to determine the endoosmotic flow (μ_{eof}) under the applied conditions using a run buffer of pH 2 or run buffers with high concentrations of CDs during a period up to 150 min, we calculated α as the relative retention using the equation 3 [12].

$$\alpha = \frac{\mu_{\mathrm{app1}}}{\mu_{\mathrm{app2}}} = \frac{t_2}{t_1} \qquad (3)$$

The resolution (R_s) between the enantiomers can be measured using the equation

$$R_s = 1.18 \cdot \frac{t_2 - t_1}{W_1^{1/2} + W_2^{1/2}} \qquad (4)$$

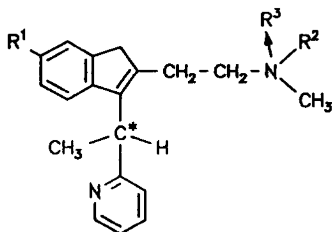
where t_1 and t_2 are the migration times and $W_1^{1/2}$ and $W_2^{1/2}$ are the peak widths at half-height of the first- and second-migrating enantiomer, respectively [13].

Achiral separation of dimethindene and metabolites

Fig. 2 shows the structures of dimethindene (**2**) and the possible metabolites **1** and **3–5**. Their achiral separation was achieved by using 50 m*M* phosphate buffer (pH 3.2) (Fig. 3). According to their basic character, secondary amines show smaller migration times than tertiary amines and weakly basic N-oxide **5** was detected last.

Chiral separation of dimethindene and metabolites

Fig. 4 shows an electropherogram of **1–5** after addition of 30 m*M* HP-β-CD to 50 m*M* phosphate buffer (pH 3.3) [14]. Under these conditions a baseline enantioseparation of **1–5** was achieved. Owing to the different selectivities in the presence of the β-CD derivatives we obtained a change in the elution order of the N-



Substance	No.	R^1	R^2	R^3
N-demethyl-dimethindene	1	H	H	
dimethindene	2	H	CH_3	
N-demethyl-6-methoxy-dimethindene	3	OCH_3	H	
6-methoxy-dimethindene	4	OCH_3	CH_3	
dimethindene-N-oxide	5	H	CH_3	O

Fig. 2. Structures of dimethindene and metabolites.

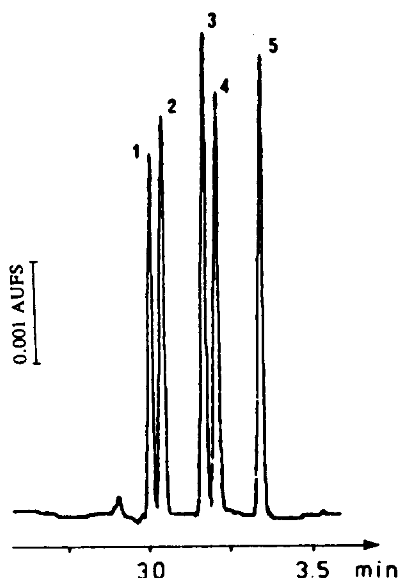


Fig. 3. Achiral separation of 1–5. Conditions: phosphate buffer (pH 3.2), 500 V/cm, 98 μA, detection at 205 nm, sample concentration 60 μg/ml.

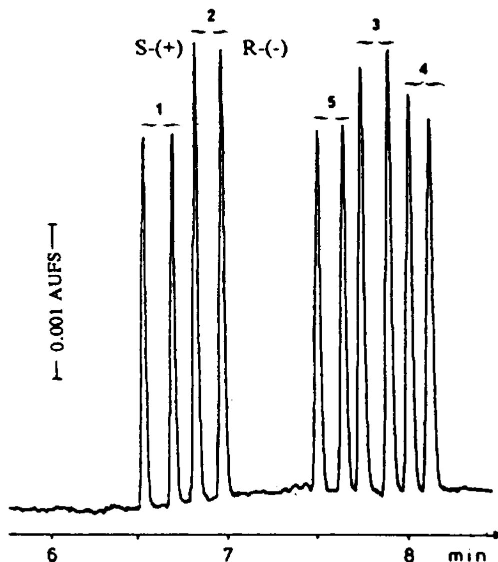


Fig. 4. Chiral separation of 1–5. Conditions: HP-β-CD 30 μg/ml, 50 m*M* phosphate buffer (pH 3.3), 400 V/cm, 42 μA, detection at 205 nm, sample concentration 30 μg/ml enantiomer.

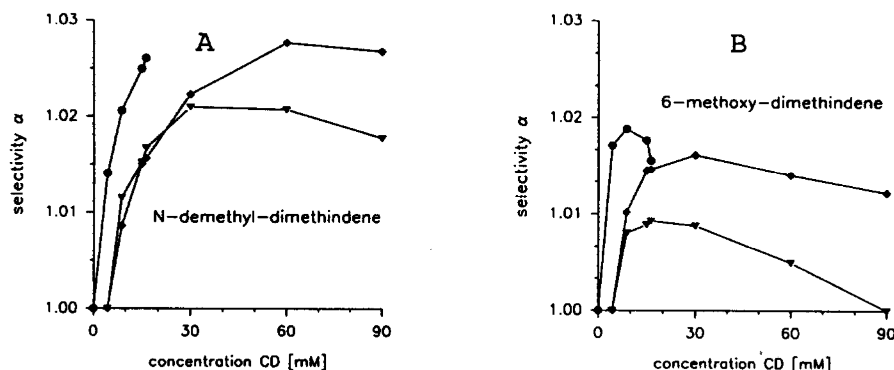


Fig. 5. Selectivity factor (α) of (A) N-demethyldimethindene (**1**) and (B) 6-methoxydimethindene (**5**) as a function of CD type and concentration. ● = β-CD; ◆ = HP-β-CD; ▼ = HE-β-CD.

oxide **5**, which now migrated between **2** and **4** [15,16].

The elution order of the dimethindene enantiomers was determined using enantiomers. (R)-(−)-**2** migrates slower, indicating the formation of a stronger diastereomer complex with β-CD derivatives.

Factors affecting chiral separations

Cyclodextrin type and concentration. Seven different CDs were tested for the resolution of **1–5**. It was found that β-CD, HE-β-CD and HP-β-CD showed enantioselectivity with respect to **1–5** (Fig. 5) [17]. Underivatized β-CD possesses the highest selectivity of all the compounds studied up to a concentration of 16.3 mM, its limit of aqueous solubility. It is known [7] that urea increases the solubility of β-CD. However, urea was not added because it was found to increase the noise of the detector signal at such high concentrations.

The selectivity of HE-β-CD and HP-β-CD is lower; however, this disadvantage is acceptable in the case of HP-β-CD, because a much higher concentration in water can be achieved owing to its high solubility. As reported for the separations of other compounds [18], an optimum concentration of 60 mM is found for **1** and **2** and 30 mM for **3–5**. The 6-methoxy substituent of **4** and **5** significantly decreases the α and R_s values (Fig. 5). Especially for **5** a very high HE-β-CD concentration resulted in a complete loss of selectivity. Non-specific hydrophobic associations have been discussed as a reason for this phenomenon [19].

The observed migration times (Fig. 6) of **1–5** in the concentration range 8.8–90 mM HP-β-CD exhibited a non-linear increase. This might be explained by the fact that high CD concentrations result in dimerization of CDs [2], leading to a loss of selectivity and hence shorter migration times.

pH of buffer solutions. Chiral separations of **1–5** were investigated separately at pH 2.0, 3.0, 3.3, 3.6, 4.0 and 4.7. Optimum selectivity was

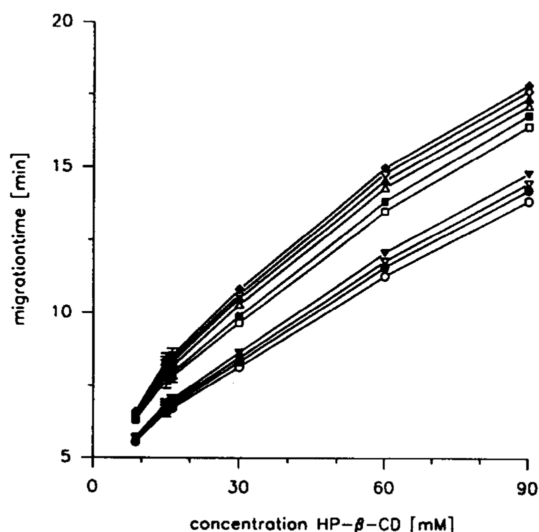


Fig. 6. Plot of the migration times of **1–5** *vs*. concentration of HP-β-CD. ○ = (+)-**1**; ● = (−)-**1**; ▽ = (+)-**2**; ▼ = (−)-**2**; □ = (+)-**3**; ■ = (−)-**3**; △ = (+)-**4**; ▲ = (−)-**4**; ◇ = (+)-**5**; ◆ = (−)-**5**.

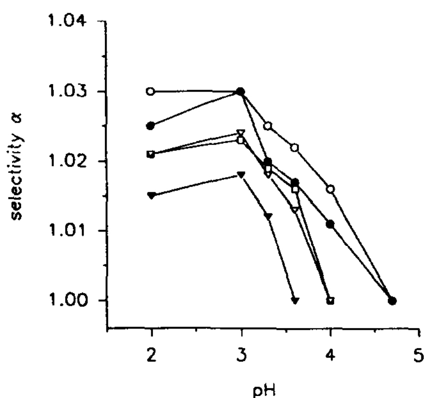


Fig. 7. Plot of selectivity factor (α) *vs.* pH of the run buffer containing 20 m*M* HP-β-CD. ○ = **1**; ● = **2**; □ = **3**; ▽ = **4**; ▼ = **5**.

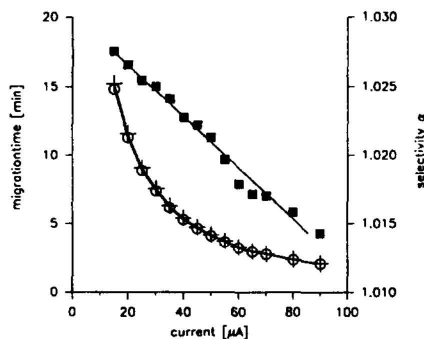


Fig. 8. Dependence of migration time and α values on the effective voltage using different constant currents. Capillary, 47 cm total length, 30 cm effective length. ■ = α for **1**; + = migration time of (−)-**1**; ○ = migration time of (+)-**1**.

found at pH 3.0 in all instances (Fig. 7). However, for the simultaneous analysis of **1–5**, pH 3.3 was used in order to avoid overlapping of the enantiomers of **3**, **4** and **5**.

Applied voltage and current. In CE, migration times can be shortened by increasing the applied voltage. Fig. 8 shows the dependence of the migration times and α values of dimethindene (**1**) on the applied voltage. Lower currents result in a higher selectivity with extremely long migration times. With a very high effective voltage of about 640 V/cm, very short migration times are achieved but, owing to Joule heating and ineffective heat dissipation, an extreme decrease in selectivity results. Good resolutions were obtained using a current of 40 μA, corresponding to an effective voltage of about 390 V/cm.

Temperature. An advantage of the Beckman P/ACE instrument over the Grom system is the

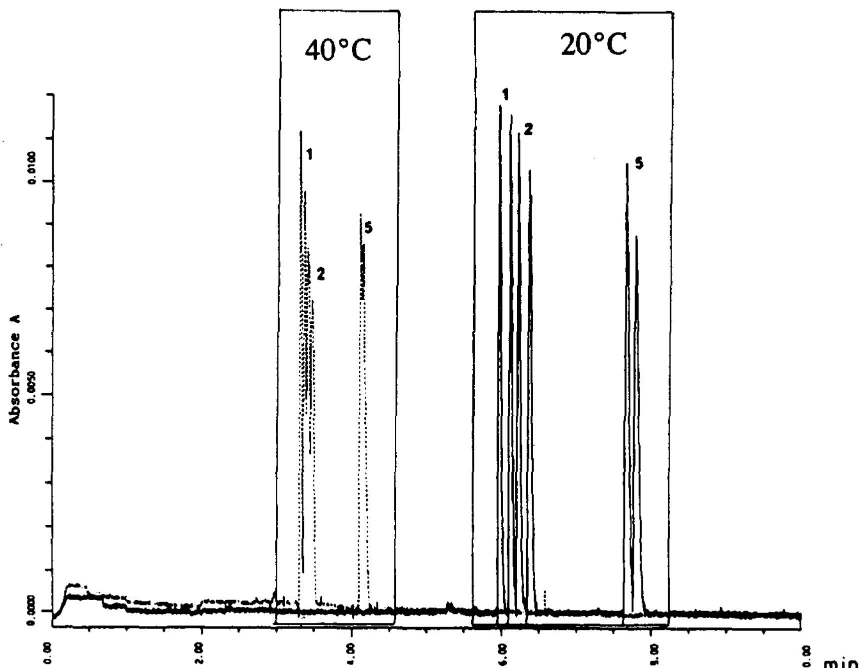


Fig. 9. Superimposition of two electropherograms showing the chiral separations of **1**, **2** and **5** at 20°C and 40°C using 50 m*M* phosphate run buffer (pH 3.3) containing 30 m*M* HP-β-CD.

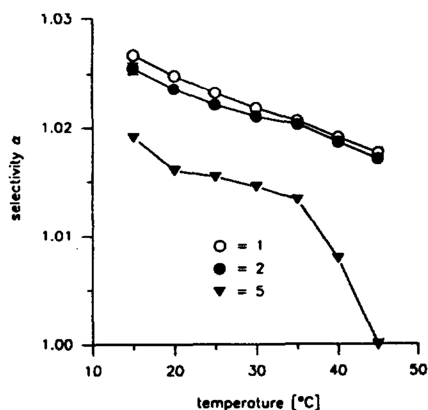


Fig. 10. Effect of the capillary temperature on chiral recognition: selectivity (α) *vs.* temperature. ○ = **1**; ● = **2**; ▼ = **5**.

use of liquid cooling to dissipate Joule heat. This allows one to work with constant capillary temperatures, independent of changes in the room temperature.

Effects of the temperature on selectivity are generally noticed in chromatography as well as in CE. The influence of temperature on the buffer pH can be used to manipulate the selectivity in CE [20].

We observed that changes in the thermostating temperature between 15 and 45°C had great effects on the migration times and enantioselectivities. Fig. 9 shows an overlay of two electropherograms of **1**, **2** and **5** at 20 and 40°C. The corresponding α values are shown in Fig. 10. The increase in the α values with a decrease in

TABLE I

RESOLUTION OF RACEMIC DRUGS

CD concentrations: α-, β-, γ-CD = 16.3 m*M*, derivatized CDs = 30 m*M* in 50 m*M* phosphate buffer (pH 3.3).

Drug	CD type	t_1 (min)	t_2 (min)	α	R_s
Ambucetamide	HP-β-CD	11.29	11.41	1.010	1.04
Carvedilol	β-CD[a]	8.88	9.05	1.019	1.50
Clenbuterol	HP-β-CL[a]	9.65	10.03	1.039	4.03
Ephedrine	β-CD[a]	7.65	7.73	1.011	0.96
Etilefrine	HP-β-CD[a]	8.35	8.68	1.038	3.82
Imafen	HP-β-CD[a]	9.12	9.28	1.019	1.60
Isoprenaline	ME-β-CD[a]	8.27	8.53	1.031	3.06
Ketamine	β-CD[a]	8.18	8.27	1.010	0.88
Lofexidine	HP-β-CD[a]	8.94	9.32	1.043	3.17
Mefloquine	HP-β-CD	13.19	14.33	1.086	7.96
Methylephedrine	ME-β-CD[a]	8.75	8.87	1.013	1.11
Metomidate	HP-β-CD	14.10	14.99	1.063	4.50
Mianserin	HP-β-CD	12.11	13.08	1.079	4.34
Nefopam	HP-β-CD	15.76	15.9	1.008	0.73
Nomifensine	HP-β-CD	14.77	15.21	1.029	2.45
Norephedrine	ME-β-CD	7.08	7.22	1.019	1.53
Norfenefrine	ME-β-CD[a]	6.79	6.99	1.029	2.59
Octopamine	HP-β-CD	8.81	8.91	1.010	0.80
Pholedrine	HP-β-CD[a]	8.65	8.87	1.026	2.29
Salbutamol	ME-β-CD[a]	7.66	7.74	1.011	1.08
Sotalol	HP-β-CD	13.97	14.13	1.011	1.20
Synephrine	ME-β-CD[a]	7.25	7.41	1.023	3.20
Zopiclone	HP-β-CD[a]	6.00	6.19	1.030	2.86

[a] One or more of the other CDs tested showed lower selectivities: β-CD, ME-β-CD, HE-β-CD and HP-β-CD.

temperature might be explained by a decrease in rotational and/or vibrational energy, increasing the fixation of the enantiomers inside or at the top of the chiral CD and, thus, increasing the enantioselectivity. Separations below 15°C were not possible with the Beckman system.

The optimum run buffer conditions for the separation of dimethindene were found to be 30 m*M* hydroxypropyl-β-cyclodextrin in 50 m*M* phosphate buffer (pH 3.3) with an effective voltage of 400 V/cm at 20°C. This method can be used to determine **1** and **2** in human urine samples after oral administration of dimethindene maleate [17]. The results obtained by this method will be reported elsewhere.

Chiral separation of different drugs

In addition to dimethindene and its possible metabolites, numerous racemic drugs with a basic nitrogen could be resolved by the addition of CDs. Table I summarizes these data, listing only those CDs which resulted in the best resolution of the drugs under the conditions applied. Some of these drugs could be separated with other CDs used in this investigation (β-CD, ME-β-CD, HE-β-CD and HP-β-CD), but with lower efficiency. With α- and γ-CD, none of these drugs could be resolved. Further, the following drugs could not be resolved after the addition of HP-β-CD to the run buffer: alimemazine, atropine, bifonazol, butetamate, camylofin, cetirizine, chlorphenoxamine, clofedanol, fenfluramine, ilmofosine, mazindol, mequitazone, metharaminol, orphenadrine, propafenone and terfenadine.

Mixtures of chiral drugs with different chemical structures can be separated and, additionally, resolved into the enantiomers in a single run, as shown in Fig. 11.

In addition to the high resolution and the

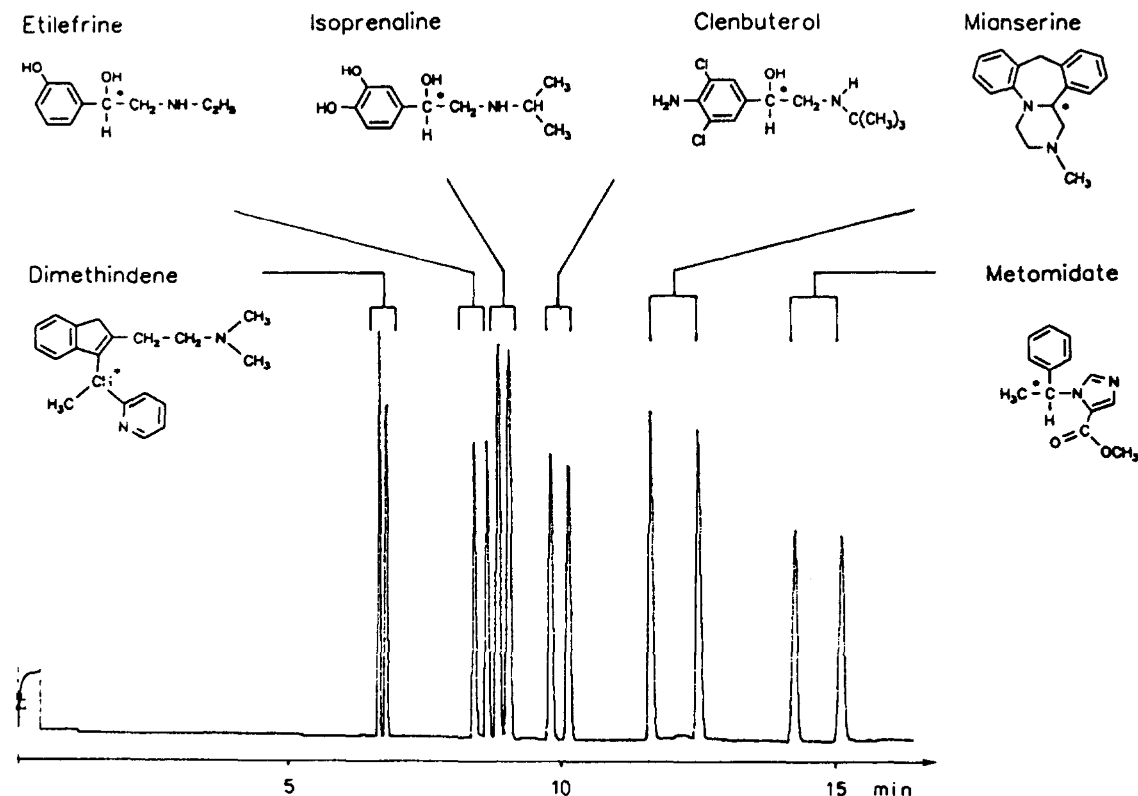


Fig. 11. Electropherogram of a mixture of six chemically different basic racemic drugs using HP-β-CD as a chiral pseudostationary phase. Run buffer: HP-β-CD, 30 mg/ml in 50 m*M* phosphate buffer (pH 3.3).

simple chemistry in CE, other advantages are low costs of buffers and pseudo-stationary chiral phases, no or minimum consumption of expensive and harmful solvents and rapidity.

CONCLUSIONS

CE proved to be a powerful tool for resolving the enantiomers of pharmaceutical drugs. The optimum separation conditions depend on the CD type and concentration, the pH of the buffer and the temperature.

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