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Separation of enantiomers of drugs by capillary electrophoresis V. Hydroxypropyl-α-cyclodextrin as chiral solvating agent

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Abstract

In an extended chiral drug screening program, enantioseparation of 86 racemic drugs was tested with hydroxypropyl-α-cyclodextrin as chiral solvating agent (CSA). A total of 34 drugs out of 86 could be resolved in this straightforward approach. The number of experiments performed under identical conditions allows a correlation of the separation factors α_m with the interaction strengths R_m. As shown for a subset of 23 drugs, the concentration of the CSA is a crucial parameter for further optimization. © 1998 Elsevier Science B.V.

Keywords: Enantiomer separation; Mobile phase separation; Drugs, chiral; Cyclodextrins

1. Introduction

For the approval of new chiral drugs [1], enantiospecific methods are now required in all industrial countries [2]. Capillary electrophoresis (CE) allows one to determine the enantiomers directly from an aqueous solution [3,4]. As observed from our database Chirbase/CE (Ver. 1/97), capillary zone electrophoresis (CZE) [5] and micellar electrokinetic chromatography (MEKC) [6,7] are most widely used for enantiomer separation by CE, accounting for 74% and 21%, respectively, of the original articles. Including reports on several modes in the same article, other modes comprise 10% of all articles.

In order to systematically explore structure–enantioselectivity relationships, we established the German–Chinese Chiral Drug Screening Program [8]. In the past few years, this long-term project has yielded a growing number of comparable data for marketed drugs. Here we report on the findings in CZE for 86 enantiomeric pairs of drugs upon addition of hydroxypropyl-α-cyclodextrin (HP-α-CD) to the run buffer.

2. Experimental

All experiments were carried out on a Bio-Focus 3000 automatic CE system (Bio-Rad Laboratories, Hercules, CA, USA), equipped with variable-wavelength detection operated at 200 or 210 nm. Operating parameters were as follows: injection: 15 kV for 3 s; analysis: 15 kV +→−; capillary temperature: 25°C. Fused-silica capillaries (0.05 mm I.D.× 0.375 mm O.D.) were obtained from Yongnian Optical Conductive Fiber Plant (Yongnian, Province

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Hebei, China). In the laboratory these were coated with polyacrylamide on the inner surface [9]. The total lengths of the capillaries used are 29 cm (alprenolol, atenolol, baclofen, bamethan, benserazide, bisoprolol, bupranolol, butamirate, butetamate, carbuterol, celiprolol, clenbuterol, clobutinol, dipivefrine, isoprenaline, metipranolol, metoprolol, norfenefrine, orciprenaline, ornidazole, oxprenolol, phenylpropanolamine, pholedrine, pirbuterol, prilocaine, procyclidine, salbutamol, sotalol, synephrine, terbutaline, tocainide), 30 cm (amorolfine, bromphenamine, bupivacaine, carteolol, chloroquine, chlorphenamine, chlorphenoxamine, disopyramide, dobutamine, doxylamine, flecainide, gallopamil, ketamine, mepindolol, orphenadrine, oxybutynin, phenoxybenzamine, pindolol, propafenone, propranolol, sulpiride, talinolol, tropicamide, verapamil), 36 cm (azelastine, biperiden, carvedilol, clidinium bromide, meclozine, mequitazine, ofloxacin, zopiclone) and 32 cm (alimemazine, atropine, benproperine, carazolol, cicletanine, dimetindene, fendiline, homatropine, ipratropium bromide, isothipendyl, mefloquine (erythro form), metaclazepam, naftidrofuryl, nefopam, nicardipine, oxomemazine, promethazine, reproterol, tetryzoline, theodrenaline, tioconazole, trihexyphenidyl, trimipramine), respectively. The effective length results from total length minus 4.5 cm.

The plain run buffer contained 100 mmol/l sodium dihydrogenphosphate and was adjusted to pH 2.5. HP-α-CD was added to the plain buffer in concentrations of 30 and 45 mmol/l, respectively. The analytes were dissolved in the run buffer to give a sample concentration of 0.1 mg/ml.

HP-α-CD (Fig. 1) was kindly donated by Wacker (Munich, Germany), batch 180292. According to the manufacturer, the substitution degree per glucose unit is 61.9%. In detail, 36.9% of the hydroxy groups in position 2, 15.8% of those in position 3 and 9.2% of those in position 6 are substituted. The drug samples (Figs. 2 and 3, respectively) were donated by the following manufacturers: Allergan, Ankerpharm, Arzneimittelwerk Dresden, ASTA Medica, Astra Chemicals, Bayer, Boehringer Mannheim, Chephasaar, Ciba-Geigy, Dispersa, Dolorgiet, Durachemie, Gödecke, Hexal, Intersan, Jenapharm, Kali-Chemie, Klinge, Knoll, Kreussler, Krewel, Mann, Medice, E. Merck, 3M Medica, Pfizer, Pharmacia, Rhône-Poulenc Rorer, Robugen, Roche, Röhm Pharma, Schering, Sigma, Thiemann, Wellcopharm and Zyma. Internal purity standards of the manufacturers apply to all drug samples. All other chemicals were analytical grade.

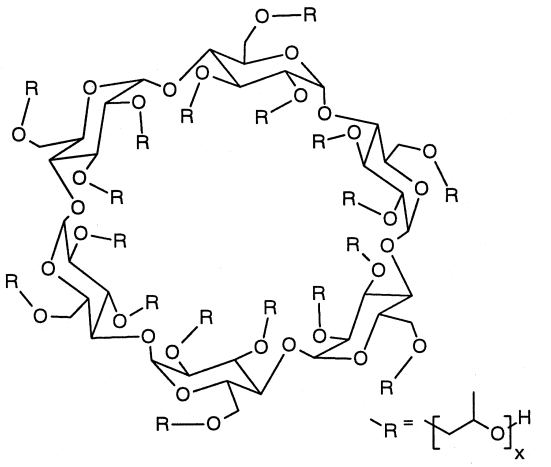

Fig. 1. Structures of the chiral solvating agent, HP-α-CD. Substitution pattern as outlined in Section 2.

3. Results and discussion

The conditions chosen for this study, pH 2.5 and migration towards the cathode, were applicable to a total of 86 entries out of 123 drugs tested. For each analyte, the migration time was determined in plain phosphate buffer as well as in 45 mmol/l chiral solvating agent (CSA) containing buffer. These data allowed to calculate the separation of the analyte enantiomers and also the retardation of the analyte due to the presence of the CSA. All data are listed in Table 1.

The electroosmotic flow (EOF) was close to zero, since uncharged compounds (e.g., dimethylsulfoxide) could not be detected within 2 h under the conditions applied. Based on this observation, a migration separation factor (α_m) was computed in a straightforward manner from the migration times of the first [$t_{m(1)}$] and second [$t_{m(2)}$] eluted enantiomer, respectively, see Eq. (1). As long as the EOF can be neglected, this parameter directly reflects the ratio of electrophoretic mobilities (μ), see Eq. (2).

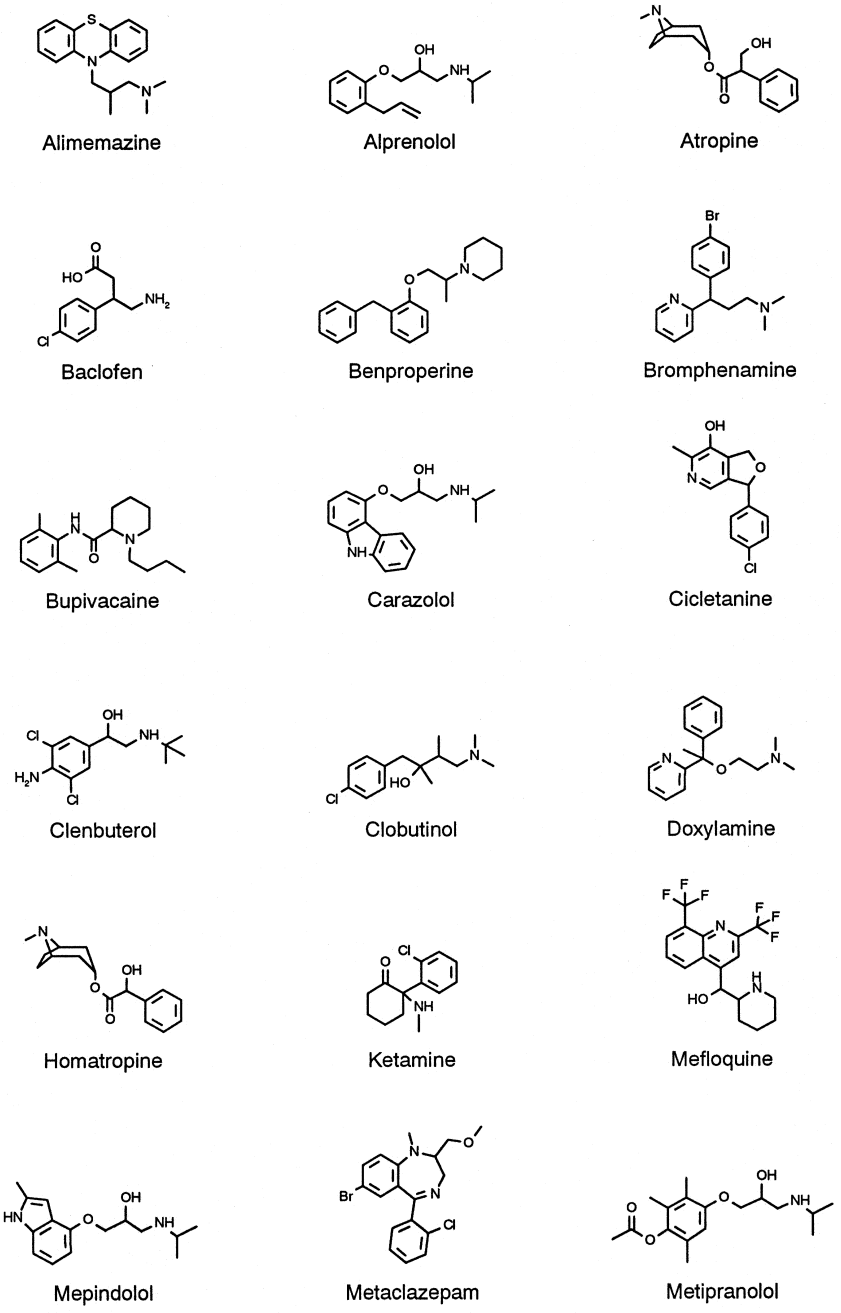

Fig. 2. Structures of the drug samples separated into enantiomers.

$$\alpha_m = t_{m(2)}/t_{m(1)} \quad (1)$$

$$t_{m(2)}/t_{m(1)} = \mu_{(1)}/\mu_{(2)} \quad (2)$$

Notably, this definition of α_m bears the advantage of not being influenced by the peak shape, thus revealing the degree of enantiodiscrimination, disre-

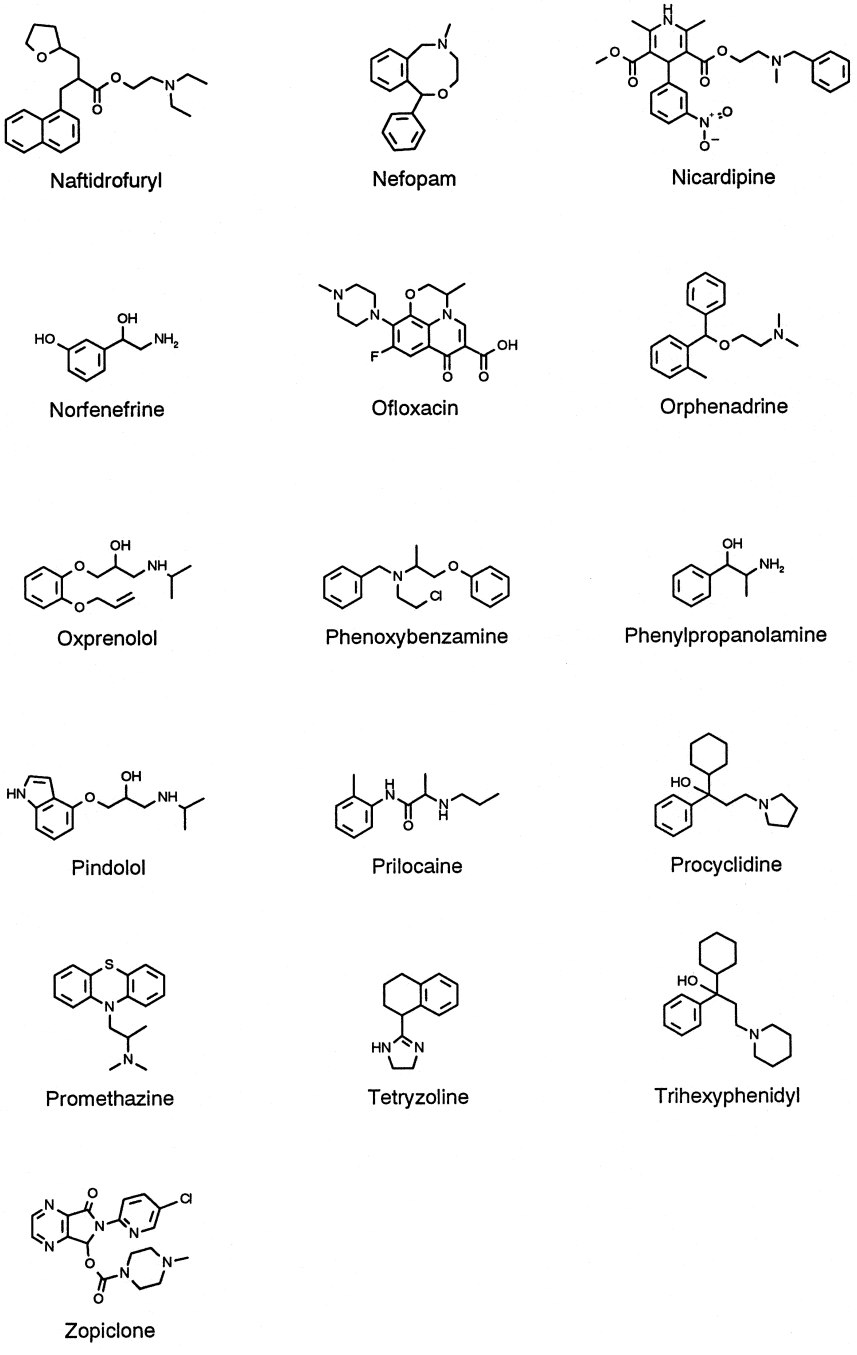

Fig. 2. (*continued*)

garding of the peak resolution achieved. Furthermore, α_m is deemed to be independent of capillary length, electric field strength etc., and therefore a good measure for the comparison of the chiral recognition of different racemates by the CSA.

A migration retardation factor (R_m) was computed

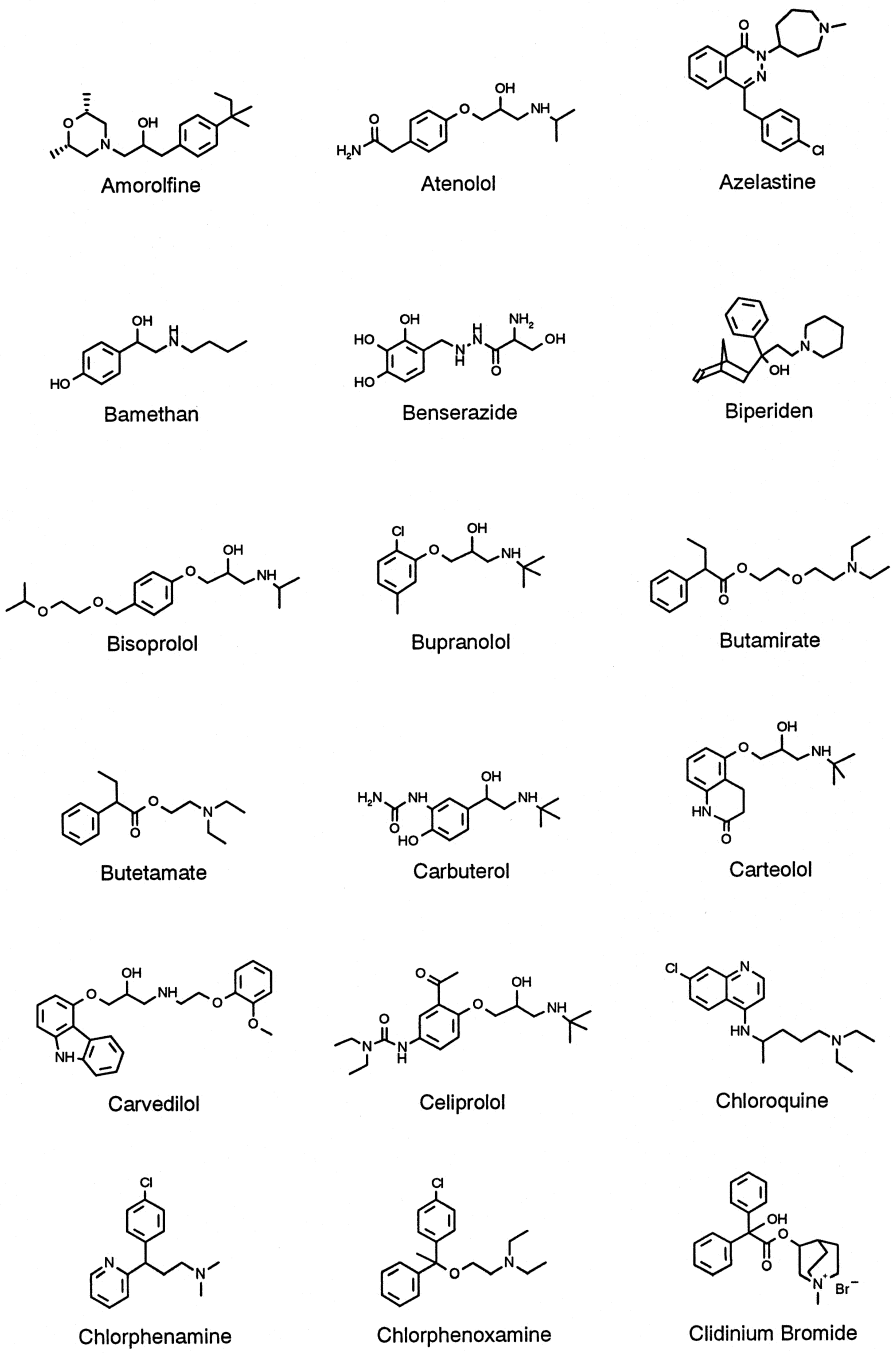

Fig. 3. Structures of the drug samples not separated into enantiomers.

accordingly from the migration times in plain phosphate buffer [$t_{m(plain)}$] and in CSA containing buffer [$t_{m(CSA)}$]; if the enantiomers were resolved, the second peak was used, see Eq. (3).

$$R_m = t_{m(CSA)}/t_{m(plain)} \quad (3)$$

Although limited to the mobility ratio of free and host-bound analyte, respectively, the retardation ob-

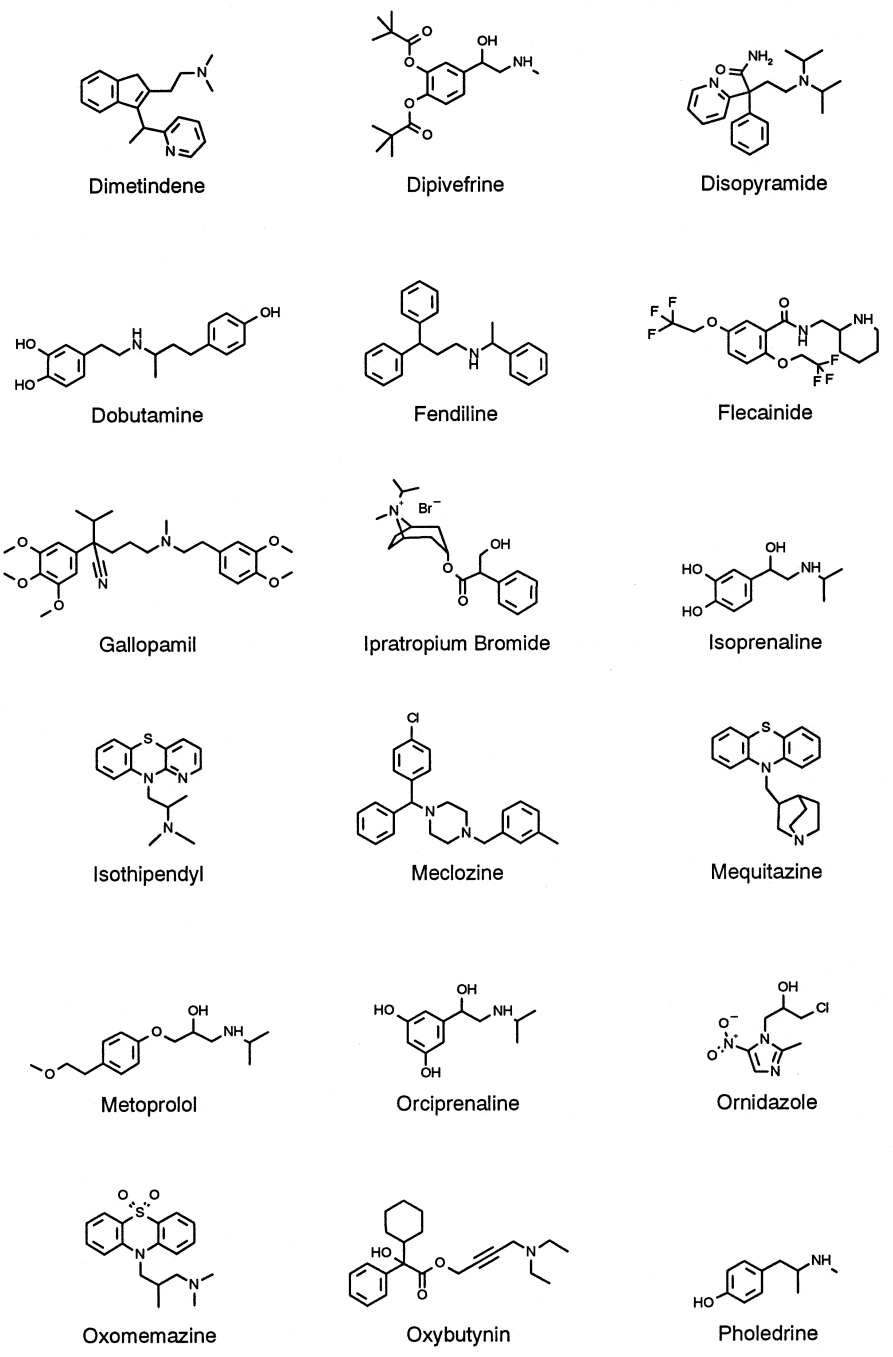

Fig. 3. (*continued*)

served provides valuable information on the strength of the host–guest interaction. Changes in the viscosity of the buffer upon CSA addition may also affect the migration times, but for all analytes to the same extent.

One of the main factors governing the degree of

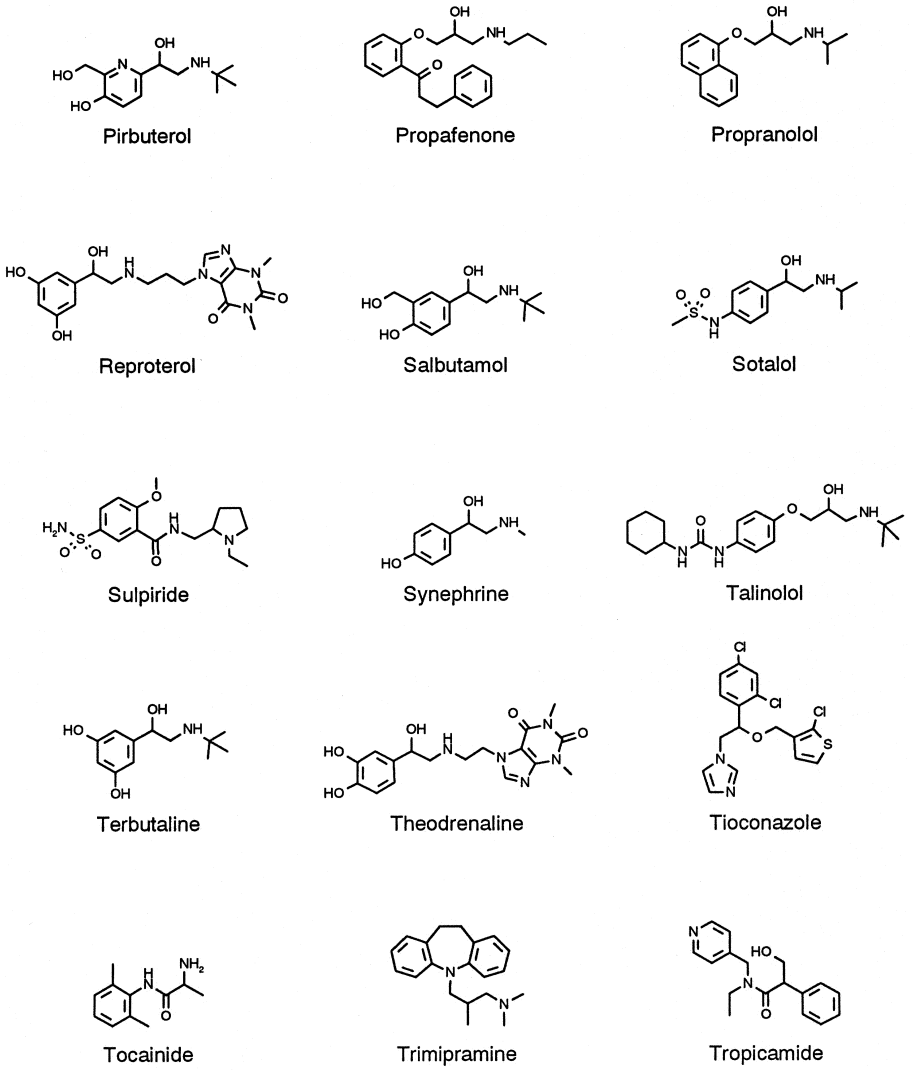

Fig. 3. (*continued*)

enantioseparation in CZE is the CSA concentration, the optimum concentration depending on the affinity constants of both enantiomers to the chiral selector [10,11] at a given pH [11]. If either too small or too high a CSA concentration is used, the separation deteriorates or even vanishes. In order to match the ''CSA concentration window'' of as many analytes as possible, a constant CSA concentration of 45 mmol/l was chosen throughout the first screening step in this study with HP-α-CD. A total of 34 drug racemates, i.e., 40% of all analytes investigated, were resolved in this straightforward approach. Fig. 4 shows two examples of the electropherograms produced.

A sufficiently high migration separation factor, $\alpha_m > 1.015$, was reached for 24 analytes. For those enantiomeric pairs that were less well separated or even unseparated, satisfactory separation factors may be achieved by adjusting the CSA concentration. A set of 23 drugs was therefore investigated at a 30

Table 1
Migration times and parameters derived thereof, for 86 analytes

Compound name	$t_{m(plain)}$ (min)	$t_{m(1)}$ (min)	$t_{m(2)}$ (min)	α_m	R_m
Alimemazine	5.18	11.92	12.13	1.018	2.342
Alprenolol	5.90	16.64	17.23	1.035	2.920
Amorolfine	5.57	14.37		1	2.580
Atenolol	6.14	7.67		1	1.249
Atropine	5.27	8.60	8.73	1.015	1.657
Azelastine	6.68	15.53		1	2.325
Baclofen	5.20	16.82	17.31	1.029	3.329
Bamethan	5.42	9.76		1	1.801
Benproperine	5.50	10.81	10.94	1.012	1.989
Benserazide	5.20	5.62		1	1.081
Biperiden	7.01	14.96		1	2.134
Bisoprolol	6.81	10.97		1	1.611
Bromphenamine	2.71	8.48	8.57	1.011	3.162
Bupivacaine	4.81	7.60	7.68	1.011	1.597
Bupranolol	5.49	12.57		1	2.290
Butamirate	5.41	17.29		1	3.196
Butetamate	5.04	17.08		1	3.389
Carazolol	5.66	10.88	10.99	1.010	1.942
Carbuterol	5.81	7.41		1	1.275
Carteolol	5.10	7.82		1	1.533
Carvedilol	7.12	15.58		1	2.188
Celiprolol	7.05	11.52		1	1.634
Chloroquine	2.81	5.24		1	1.865
Chlorphenamine	2.56	7.69		1	3.004
Chlorphenoxamine	4.47	14.93		1	3.340
Cicletanine	5.50	14.35	14.67	1.022	2.667
Clenbuterol	6.35	7.28	7.45	1.023	1.173
Clidinium bromide	6.42	10.59		1	1.650
Clobutinol	4.85	17.75	17.98	1.013	3.707
Dimetindene	3.17	4.94		1	1.558
Dipivefrine	6.10	10.37		1	1.700
Disopyramide	3.96	6.37		1	1.609
Dobutamine	5.34	9.42		1	1.764
Doxylamine	2.53	4.58	4.74	1.035	1.874
Fendiline	6.05	11.77		1	1.945
Flecainide	5.27	12.19		1	2.313
Gallopamil	6.27	10.64		1	1.697
Homatropine	5.05	7.67	7.91	1.031	1.566
Ipratropium bromide	5.41	8.96		1	1.656
Isoprenaline	5.11	7.18		1	1.405
Isothipendyl	4.80	11.04		1	2.300
Ketamine	3.76	7.13	7.36	1.032	1.957
Meclozine	6.62	17.82		1	2.692
Mefloquine	6.31	11.65	13.39	1.149	2.122
Mepindolol	4.68	8.14	8.26	1.015	1.765
Mequitazine	6.16	14.22		1	2.308
Metaclazepam	5.67	9.78	10.32	1.055	1.820
Metipranolol	6.11	11.21	11.48	1.024	1.879
Metoprolol	5.57	14.41		1	2.587
Naftidrofuryl	6.15	10.63	10.91	1.026	1.774
Nefopam	4.90	8.96	9.29	1.037	1.896
Nicardipine	6.81	11.06	11.37	1.028	1.670
Norfenefrine	3.95	5.41	5.48	1.013	1.387

Table 1. Continued

Compound name	$t_{m(plain)}$ (min)	$t_{m(1)}$ (min)	$t_{m(2)}$ (min)	α_m	R_m
Ofloxacin	6.42	8.00	8.37	1.046	1.304
Orciprenaline	5.17	6.97		1	1.348
Ornidazole	13.38	23.91		1	1.787
Orphenadrine	4.46	10.43	10.90	1.045	2.444
Oxomemazine	5.28	9.69		1	1.835
Oxprenolol	5.17	10.77	10.95	1.017	2.118
Oxybutynin	5.43	12.51		1	2.304
Phenoxybenzamine	4.63	9.37	9.84	1.050	2.125
Phenylpropanolamine	3.94	6.21	6.40	1.031	1.624
Pholedrine	4.10	6.39		1	1.559
Pindolol	4.55	7.78	7.95	1.022	1.747
Pirbuterol	3.83	4.00		1	1.044
Prilocaine	6.01	8.05	8.32	1.034	1.384
Procyclidine	3.93	16.35	16.67	1.020	4.242
Promethazine	5.06	12.29	12.41	1.010	2.453
Propafenone	5.42	12.78		1	2.358
Propranolol	4.73	10.55		1	2.230
Reproterol	7.21	8.94		1	1.240
Salbutamol	5.42	6.88		1	1.269
Sotalol	5.33	6.58		1	1.235
Sulpiride	4.71	6.32		1	1.342
Synephrine	4.26	6.39		1	1.500
Talinolol	6.99	15.29		1	2.187
Terbutaline	5.48	7.06		1	1.288
Tetryzoline	4.24	6.78	6.85	1.010	1.616
Theodrenaline	6.96	8.45		1	1.214
Tioconazole	5.48	15.18		1	2.770
Tocainide	4.50	5.99		1	1.331
Trihexyphenidyl	6.23	13.57	13.77	1.015	2.210
Trimipramine	5.49	11.82		1	2.153
Tropicamide	4.73	8.43		1	1.782
Verapamil	6.74	12.98		1	1.926
Zopiclone	6.98	10.33	10.64	1.030	1.524

$t_{m(plain)}$: Migration time in plain phosphate buffer.
$t_{m(1)}$: Migration time of the first eluted enantiomer in 45 mmol/l HP-α-CD containing buffer.
$t_{m(2)}$: Migration time of the second eluted enantiomer in 45 mmol/l HP-α-CD containing buffer.
α_m: Migration separation factor.
R_m: Migration retardation factor.

mmol/l CSA concentration (Table 2). This change in the CSA concentration had a varying impact on the different drugs selected, vide infra.

Among the drugs separated, mefloquine has an exclusively high migration separation factor. This may be explained by its high rigidity, associated with the fact that most atoms are either ring members or part of trifluoromethyl groups with a local C_{3v} symmetry. Only the rotation around two single bonds will effectively change the overall shape of the molecule. Provided that, in the presence of the chiral host, the two enantiomers will undergo such a conformation change to a different extend, their interaction strength with this host will differ accordingly. Future thermodynamic studies may provide a further clue to this interesting question. The situation is almost similar for metaclazepam, although to a smaller extend, as judged from the lower α_m value. Electropherograms of the two compounds are shown in Fig. 4. Whereas here a benzene ring is at least

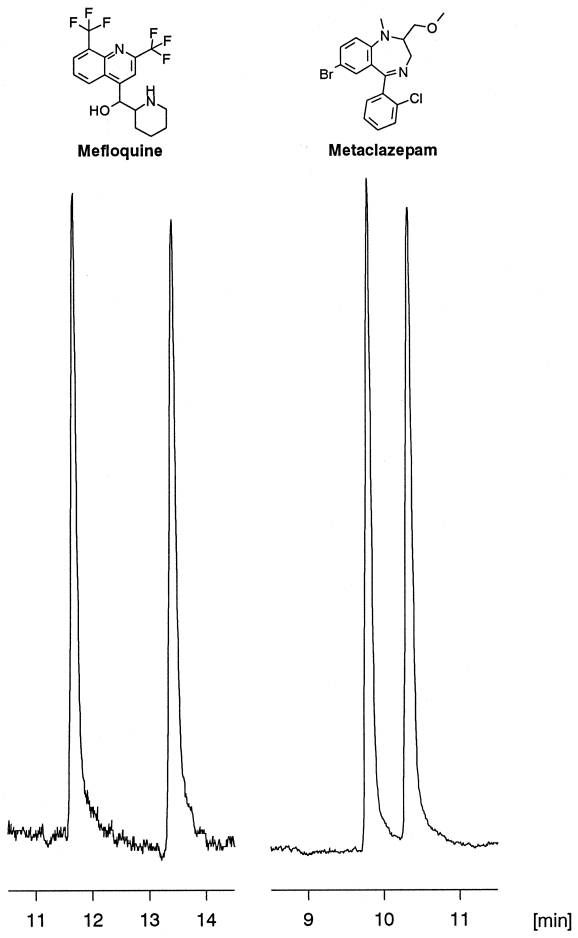

Fig. 4. Electropherograms of mefloquine and metaclazepam enantiomers with 45 mmol/l HP-α-CD. For other conditions see Section 2.

partially incorporated into the cyclodextrin cavity, compounds with more polar, substituted phenyl rings will switch to another binding mode, by dipping in their alkyl groups, e.g., the *tert.*-butyl group in clenbuterol. Ongoing studies by ^{1}H NMR and microcalorimetry will answer some of these questions in due time.

Given CE data alone, the molecular structure of the host–guest complexes remains unknown. One should bear in mind that the different hydroxypopyl substituents lead to numerous conformations of the host structure. Moreover, the CSA applied consists of a whole library of different substitution patterns. Consequently, a fairly good success for an analytical purpose is paralleled by great difficulties in finding definite structure–enantioselectivity relationships.

On the other hand, the huge number of experiments performed may serve as a robust basis for a statistical overview. For example, it seems worthwhile to deal with the relation between the separation and the retardation, particularly in the optimization experiments (Fig. 5a). Only a few compounds with low migration retardation factor ($R_m < 1.5$) were separated ($\alpha_m > 1$). As discussed, the retardation upon addition of the CSA may be used as an approximate measure of the interaction strength, limited by the ratio of absolute mobilities of both free and host-bound analyte. Apparently, small or vanishing retardation are a hallmark of weak affinity, and therefore associated with a poor enantioseparation. A maximum probability of separation is reached at medium retardation ($R_m \approx 2$, only fractions containing at least three analytes are considered). It remains to be seen with excessively large data collections as to what extent the probability levels off beyond the optimum retardation, see Fig. 5b; R_{suc} was calculated according to Eq. (4).

$$R_{suc} = \text{Analytes}_{separated}/\text{Analytes}_{total} \quad (4)$$

Indeed, there is a significant increase of success ratio and medium migration separation factor to be seen in the low R_m domain. At higher regions of R_m, however, there is only an insufficient number of entries present, rendering the observed trends insignificant.

A maximum difference in total mobilities of the enantiomers can be reached if about half of each enantiomer is bound to the CSA [12]. High complex formation constants, showing in strong retardation, are thus counterbalanced by choosing a lower CSA concentration, and vice versa. The second CSA concentration of 30 mmol/l tested for 23 drugs seems close enough to the initial value of 45 mmol/l to avoid the occurrence of extreme values of α_m between the two concentrations. This assumption could be verified. Most racemates not resolved at 45 mmol/l CSA are not influenced ($\Delta\alpha_m = 0$); these datapoints are omitted in Fig. 6 for clarity. Most of

Table 2
Influence of the CSA concentration on the migration separation factor of 23 analytes

Compound name	$t_{m(1)}$ (min)	$t_{m(2)}$ (min)	$\alpha_{m(30)}$	$\alpha_{m(45)}$	$\Delta\alpha_m$
Analytes with variable α_m					
Alimemazine	9.48	9.66	1.019	1.018	0.001
Atropine	7.79	7.89	1.013	1.015	−0.002
Benproperine	10.08	10.20	1.012	1.012	0.000
Carazolol	9.84	9.95	1.011	1.010	0.001
Cicletanine	11.54	11.80	1.023	1.022	0.000
Homatropine	7.03	7.22	1.027	1.031	−0.004
Mefloquine	9.93	11.36	1.144	1.149	−0.005
Metaclazepam	7.64	7.99	1.046	1.055	−0.009
Naftidrofuryl	9.32	9.53	1.023	1.026	−0.004
Nefopam	7.94	8.16	1.028	1.037	−0.009
Nicardipine	9.87	10.12	1.025	1.028	−0.003
Promethazine	10.17	10.29	1.012	1.010	0.002
Tetryzoline	6.11		1	1.010	−0.010
Trihexyphenidyl	12.82	13.00	1.014	1.015	−0.001
Trimipramine	11.20	11.28	1.007	1	0.007
Analytes with constant α_m					
Dimetindene	4.63		1	1	0
Fendiline	9.10		1	1	0
Ipratropium bromide	8.17		1	1	0
Isothipendyl	9.93		1	1	0
Oxomemazine	8.28		1	1	0
Reproterol	8.41		1	1	0
Theodrenaline	8.19		1	1	0
Tioconazole	12.10		1	1	0

$t_{m(1)}$: Migration time of the first eluted enantiomer in 30 mmol/l HP-α-CD containing buffer.
$t_{m(2)}$: Migration time of the second eluted enantiomer in 30 mmol/l HP-α-CD containing buffer.
$\alpha_{m(30)}$: Migration separation factor in 30 mmol/l HP-α-CD containing buffer.
$\alpha_{m(45)}$: Migration separation factor in 45 mmol/l HP-α-CD containing buffer.
$\Delta\alpha_m$: Change of the migration separation factor on decreasing the CSA concentration.

the strongly retarded analytes [$R_{m(45\ mmol/l\ CSA)}>2$] profit from the concentration decrease ($\Delta\alpha_m>0$), but there are also some less strongly retarded analytes [$R_{m(45\ mmol/l\ CSA)}<2$] that show a better separation at higher CSA concentration. A linear correlation for the data displayed in Fig. 6 would result in a regression coefficient of $r^2=0.32$.

Hence, without resorting to the elaborate determination of the individual complex formation constants of the two enantiomers with the CSA [13,14], just one experiment per compound at medium CSA concentration and one reference measurement without CSA may be sufficient to direct the course of further optimization for individual racemates [15].

Among the many CSAs applied in CZE, HP-α-CD is one of the less frequently used examples [14,16–18]. We are confident that the good overall success ratio achieved here will boost a more intense investigation of this ''broadband'' CSA.

Acknowledgements

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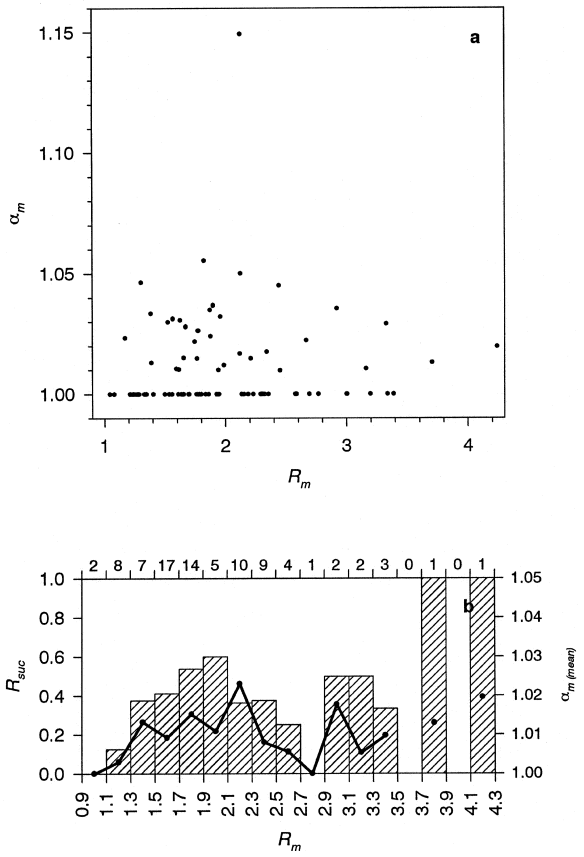

Fig. 5. Investigation of 86 drugs with HP-α-CD. (a) Plot of migration separation factor α_m versus migration retardation factor R_m. (b) Success ratio R_{suc} (bars) and mean migration separation factor $\alpha_{m(mean)}$ (line) in different slices of R_m.

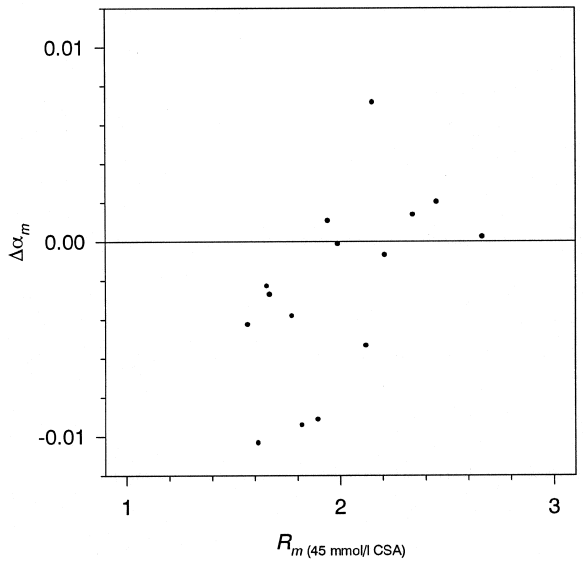

Fig. 6. Plot of $\Delta\alpha_m = \alpha_{m(30\ mmol/l)} - \alpha_{m(45\ mmol/l)}$ versus R_m at 45 mmol/l, for 15 out of 23 drugs (eight drug racemates unseparated under both conditions were omitted for clarity).

pharmaceutical companies listed and Wacker (Burghausen, Germany).

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