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Separation of enantiomers of drugs by capillary electrophoresis III. β-cyclodextrin as chiral solvating agent

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Abstract

Enantiomer separation by capillary zone electrophoresis was studied for a set of 34 chiral drugs. Keeping the concentration of β-cyclodextrin as a chiral solvating agent as constant as possible led to the separation of seven enantiomeric pairs. Carvedilol, Tetryzoline, Tropicamide and Zopiclone gave a baseline separation, Chlorphenamine, Ketamine, and Orciprenaline a partial separation. Statistical analysis revealed that the best separation factors were observed for a medium degree of interaction with the cyclodextrin. A theory explaining this effect provides a helpful guideline for further optimization.

Keywords: Enantiomer separation; Buffer composition; Drugs; Zopiclone; Cyclodextrins

1. Introduction

Enantiomers often differ in their effect to the living matter, as this is composed of chiral building blocks like amino acids, nucleic acids and sugars [1,2]. During the past decade, this fact was widely recognized, leading to a more strict approval policy of chiral drugs in all industrial countries [3]. Consequently, enantiomerically specific analytical methods are required for both, research and production monitoring of the enantiomeric composition.

In addition to high performance liquid chromatography (HPLC) and gas chromatography (GC), capillary electrophoresis (CE) has turned out as one of the most promising methods, in terms of resolution power, ease of sample preparation and analysis time. Different operation modes of CE have been successfully applied to the separation of enantiomers on an analytical scale, as recorded in our graphical molecular database Chirbase/CE [4]. Whereas isotachophoresis (ITP) [5,6], capillary gel electrophoresis (CGE) [7,8], micellar electrokinetic chromatography (MEKC) [9,10] and capillary electrochromatography (CEC) [11,12] are of minor importance, capillary zone electrophoresis (CZE) [13,14] contributes to 72% of all documents in Chirbase/CE. By far the most widely used chiral solvating agents (CSAs) are cyclodextrins, especially native β-cyclodextrin and derivatives thereof; they are applied in 99 out of 138 relevant papers.

As part of a more extented screening of the separation of racemic drugs in CZE by different CSAs [15,16], we report here on the suitability of native β-cyclodextrin (Fig. 1) [17] for a subset of 34 drugs that have one stereogenic center in their molecular structure. The primary aim of this study was not to separate as many compounds as possible, but to evaluate on a statistical basis the behaviour of

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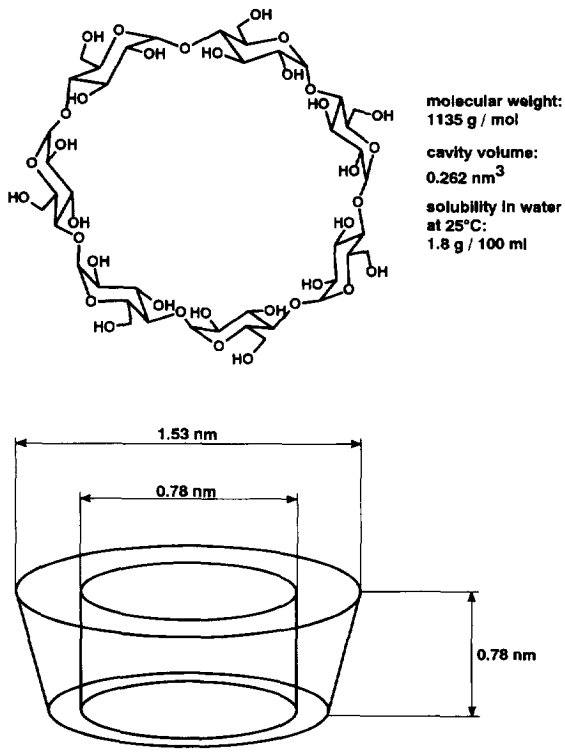


Fig. 1. β-Cyclodextrin: structure, shape, properties.

these compounds while keeping the operation conditions as constant as possible. Eventually, these data may serve as an unbiased guideline for choosing the most promising CSA among the many alternatives offered today.

2. Experimental

All experiments were carried out on a Bio-Focus 3000 automatic capillary electrophoresis system (Bio-Rad Laboratories, Hercules, CA, USA) equipped with a variable wavelength detector operated at 200 nm and an polyacrylamide coated capillary from the same manufacturer. β-Cyclodextrin was obtained from Wacker Chemie (Munich, Germany). Analytical samples of the chiral drugs were supplied by different pharmaceutical companies: 3M Medica (Flecainide, Orphenadrine, Pirbuterol, Salbutamol), Ankerpharm (Ipratropium Bromide, Tropicamide), Astra Chemicals (Prilocaine, Tocainide), Arzneimittelwerk Dresden (Trimipramine), Boehringer Mannheim (Carvedilol), Durachemie (Pindolol), Gödecke (Carbuterol, Ketamine), Hexal (Dobutamine, Naftidrofuryl, Sotalol), Jenapharm (Atenolol), Klinge (Carazolol), Knoll (Propafenone), Krewel (Butetamate, Nefopam), Mann (Metipranolol, Ofloxacin), Pfizer (Tetryzoline), Röhm Pharma (Chlorphenamine), Rhône-Poulenc Rorer (Bupivacaine, Mequitazine, Zopiclone), and Roche (Benserazide, Clidinium Bromide). All other chemicals were analytical grade.

The run buffer was prepared from a 0.1 $mol \cdot l^{-1}$ solution of sodium dihydrogenphosphate (NaH_2PO_4) and adjusted to the pH of 2.5 with phosphoric acid (H_3PO_4). As a chiral solvating agent (CSA), β-cyclodextrin was dissolved in the plain phosphate buffer, to give a 0.015 $mol \cdot l^{-1}$ solution.

Stock solutions of the bulk drug samples (1 $g \cdot l^{-1}$) were prepared in deionized and distilled water. These were diluted 10 fold with a 1:1 mixture of purified water and the run buffer, to give the sample solutions, that were transferred to the capillary by electromigration. The injection was carried out by applying a voltage of 8 kV for 6 s.

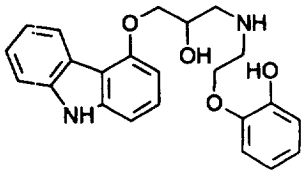
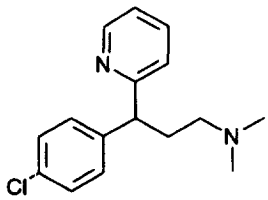
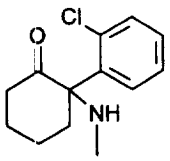
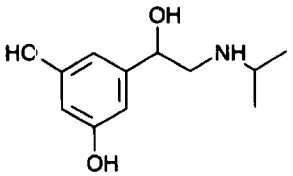
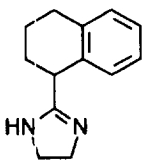
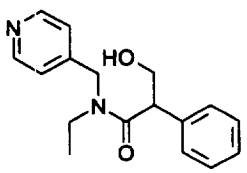
3. Results

Apart from random deviations, the separation conditions were kept constant for all compounds investigated, see Experimental and Table 1. Under these conditions, seven enantiomeric pairs could be separated. In Table 1, the analytes separated into enantiomers are listed in alphabetical order of their names. All drug names used are international nonproprietary names assigned to the pharmaceuticals by the World Health Organization (WHO).

Following the suggestion of Heuermann and Blaschke [18], we defined a *migration separation factor* (α_m) [16], see Eq. 1, in analogy to the separation factor (α) frequently used in chromatography.

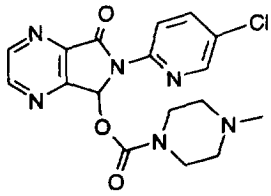
$$\alpha_m = t_{m(2)}/t_{m(1)} \quad (1)$$

Table 1
Compounds successfully separated into the enantiomers, under standard conditions

Compound structure	Pharmaceutical name	$t_{m(plain)}$ [min]	$t_{m(1)}$ $t_{m(2)}$ [min]	α_m
	Carvedilol	11.07	15.39 15.75	1.023
	Chlorphenamine	6.60	7.75 7.86	1.014
	Ketamine	8.60	8.44 8.52	1.009
	Ociprenaline	9.95	9.25 9.39	1.015
	Tetryzoline	7.57	7.33 7.50	1.021
	Tropicamide	8.60	10.89 11.33	1.040

(Continued on p. 336)

Table 1 (*continued*)

Compound structure	Pharmaceutical name	$t_{m(plain)}$ [min]	$t_{m(1)}$ $t_{m(2)}$ [min]	α_m
	Zopiclone	9.44	9.66 10.02	1.037

$t_{m(plain)}$ = migration time in plain phosphate buffer.
$t_{m(1)}$, $t_{m(2)}$ = migration times in cyclodextrin containing phosphate buffer; first and second detected enantiomer, respectively.
α_m = migration separation factor of the enantiomers.
Conditions: Instrument: Bio-Rad Bio-Focus 3000; Capillary: fused silica, polyacrylamide coated, 44.5 cm total length, 40.0 cm effective length, 50 μm inner diameter; Sample concentration: 0.1 mg/ml; Buffer: phosphate, 100 mmol/l, pH 2.5; CSA: β-cyclodextrin, 15 mmol/l; Injection: 6 s, 8 kV; Run: 14 kV+→−; Detection: 200 nm/0.005 AUF; Capillary temperature: 30°C.

where $t_{m(1)}$ and $t_{m(2)}$ are the migration times of the first and the second eluted enantiomer, respectively.

Assuming that the electroosmotic flow (EOF) is approaching zero, as it seems to be justified in this study, α_m is equivalent to the ratio (α_μ) of the electrophoretic mobilities of the enantiomers [18], see Eq. 2,

$$\alpha_\mu = \mu_1 / \mu_2 \tag{2}$$

where μ_1 and μ_2 are the electrophoretic mobilities of the first and second eluted enantiomer, respectively.

It is beyond the scope of this article to outline the relationship between α_m and the complex formation constants (K_1) and (K_2) of the two drug enantiomers with the cyclodextrin host. Provided that the EOF can be neglected, α_m is a readily accessible measure for the discrimination phenomenon.

Similar to the effective mobility difference of the enantiomers $\Delta\mu$ calculated by Wren and Rowe [19,20], see Eq. 3,

$$\Delta\mu = \mu_{eff(1)} - \mu_{eff(2)} \tag{3}$$

α_m is expected to have a maximum at a certain CSA concentration, namely at

$$c_{opt} = (\mu_u / \mu_c)^{1/2} \cdot (K_1 \cdot K_2)^{-1/2} \tag{4}$$

where c_{opt} is the CSA concentration producing the largest α_m value, and μ_u and μ_c are the electrophoretic mobilities of the uncomplexed and complexed forms of the analyte, respectively.

Complete resolution of the enantiomers was observed for Carvedilol ($\alpha_m = 1.023$), Tetryzoline ($\alpha_m = 1.021$), Tropicamide ($\alpha_m = 1.040$) and Zopiclone ($\alpha_m = 1.037$, electropherogram see Fig. 2). The migration separation factors achieved for Chlorphenamine, Ketamine and Orciprenaline were not sufficient for a baseline separation. Earlier reports on the separation of Carvedilol, Ketamine [18] and Chlorphenamine [21,22] with β-cyclodextrin could thus be confirmed. 27 Racemates failed to separate under the unified conditions applied. These are compiled in alphabetical order of their names in Table 2, along with their molecular structures and the migration times in plain phosphate buffer and in CSA containing buffer.

As a preliminary tool to elucidate the impact of the cyclodextrin on the migration velocity, we defined a *migration retardation factor* (R_m) as the ratio of the migration time (if resolved, of the second eluted enantiomer) in CSA containing buffer ($t_{m(CSA)}$) and the migration time in plain phosphate buffer ($t_{m(plain)}$), see Eq. 5 [16].

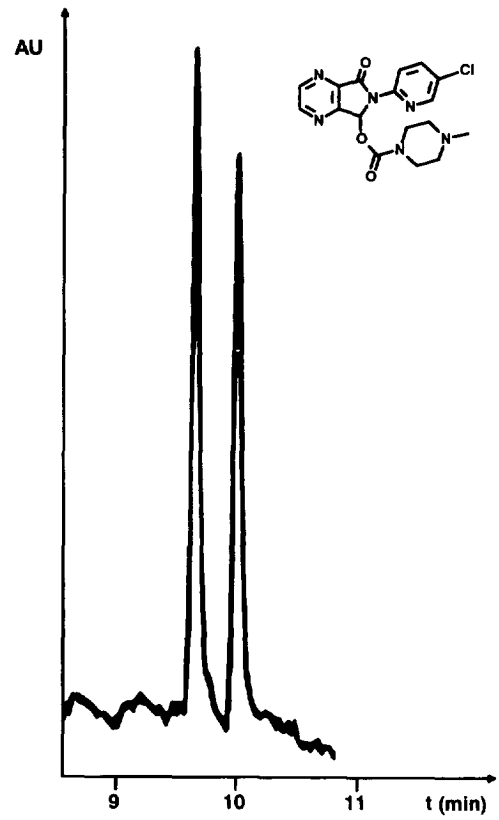


Fig. 2. Electropherogram of Zopiclone enantiomers; conditions as quoted in Table 1.

$$R_m = t_{m(CSA)}/t_{m(plain)} \quad (5)$$

R_m reflects the ratio of the electrophoretic mobility of the uncomplexed compound and the electrophoretic mobility of the second eluted enantiomer in CSA containing buffer, μ_u/μ_2. This ratio approaches μ_u/μ_c at higher CSA concentrations, as long as K_2 is greater than zero.

In practice, $t_{m(CSA)}$ and $t_{m(plain)}$ cannot be measured in one electrophoretic experiment; hence, the R_m values are subject to the limits of long-term reproducibility of the absolute migration times. Apart from systematic drifts, random deviations may become of vanishing importance as a large number of data points is investigated.

4. Discussion

The given couple of CSA concentration and buffer pH used in this survey is a priori not optimum, because different complex formation constants and pK_A values of different selectands are expected to cause significant variations in the individual optimum separation conditions. As demonstrated by Vigh and co-workers [23,24], the influence of the pH value of the background electrolyte on the resolution is at least as important as that of the selector concentration. In subsequent steps of our ongoing screening program, this parameter will also be varied.

As compared to GC, in CE solvation effects give rise to additional complexity of the interaction model. Therefore, in practice, a clue to the separation behaviour of a particular analyte is not easily found in the molecular structures of both selector and selectand. Usually β-cyclodextrin is recommended as a selector molecule for mono- or bicyclic compounds. Although, all examples separated confirm this *rule of thumb*, a characteristic common substructure cannot be found. A phenyl ring directly connected to the chiral center is contained in four of the seven separation examples, but also in Biperiden, Butetamate, Disopyramide, Nefopam, Sotalol, and other drugs that are not separated (cf. Tables 1 and 2).

At the present stage of this investigation, it may be more appropriate to elaborate a statistical description of the experiments performed. Fig. 3 shows a plot of the separation factor (α_m) versus the retardation factor (R_m). Despite the small number of compounds separated into enantiomers, this two-dimensional layout reveals a first insight. All separations are found within the range of $R_m = 0.9 - 1.5$; most of the unseparated compounds, however, are in the region of $R_m = 0.7 - 1.3$, pointing to the virtual absence of significant interaction of these analytes with the cyclodextrin selector. While it remains to be settled whether some analytes are indeed accelerated in the presence of β-cyclodextrin ($R_m = 0.7 - 1.0$), it can be seen that the medium R_m range indicates a degree of interaction between selector and selectand that is favourable for a separation.

This tendency is expressed even more clearly in

Table 2
Compounds not separated into the enantiomers, under standard conditions

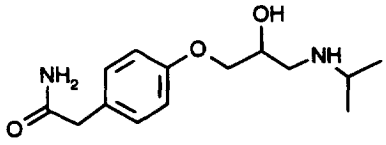
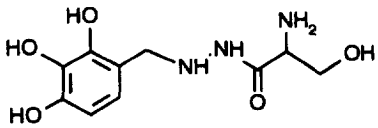
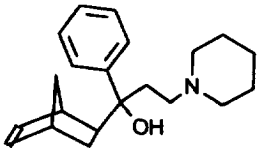
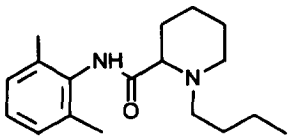
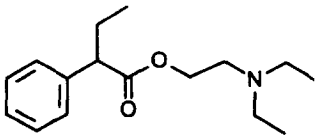
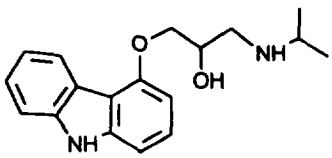
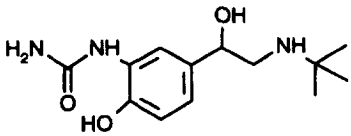
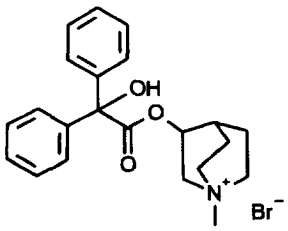
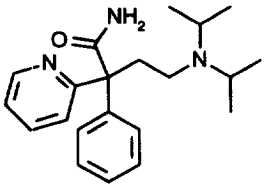
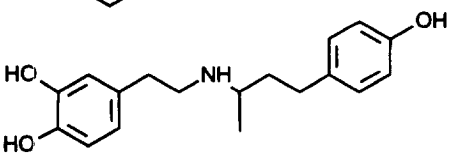
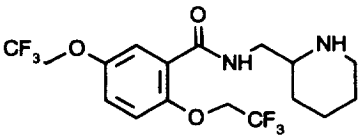
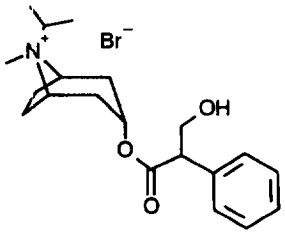
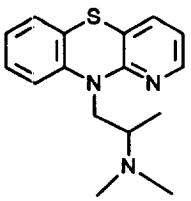
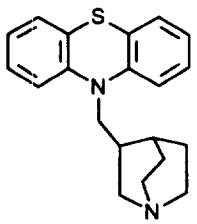
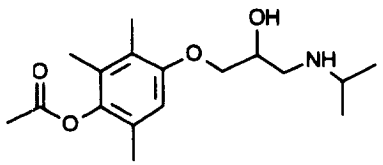
Compound structure	Pharmaceutical name	$t_{m(plain)}$ [min]	$t_{m(CSA)}$ [min]
	Atenolol	10.61	12.99
	Benserazide	9.02	8.58
	Biperiden	11.52	14.74
	Bupivacaine	11.87	8.69
	Butetamate	10.31	12.93
	Carazolol	10.28	11.16
	Carbuterol	10.50	10.19
	Clidinium bromide	10.05	17.05

Table 2 (*continued*)

Compound structure	Pharmaceutical name	$t_{m(plain)}$ [min]	$t_{m(CSA)}$ [min]
	Disopyramide	9.35	8.47
	Dobutamine	11.27	18.57
	Flecainide	11.60	14.70
	Ipratropium bromide	10.16	15.95
	Isothipendyl	8.83	8.52
	Mequitazine	8.51	20.00
	Metipranolol	11.13	11.92

(*Continued on p. 340*)

Table 2 (*continued*)

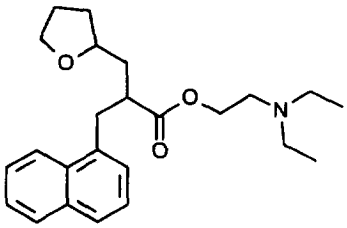
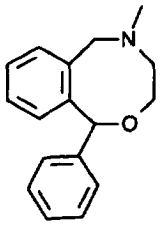
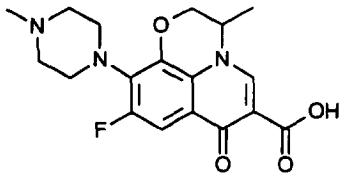
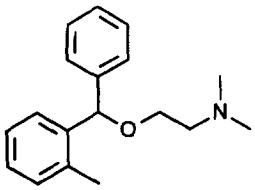
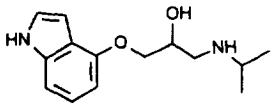
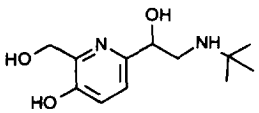
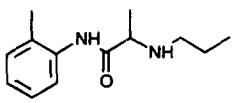
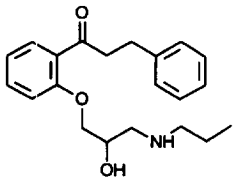
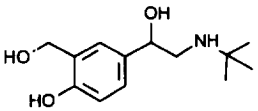
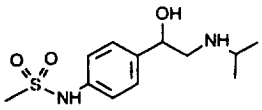
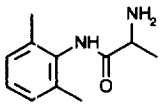
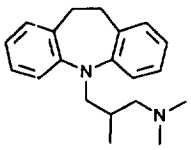
Compound structure	Pharmaceutical name	$t_{m(plain)}$ [min]	$t_{m(CSA)}$ [min]
	Naftidrofuryl	11.56	18.17
	Nefopam	9.15	13.06
	Ofloxacin	10.30	8.87
	Orphenadrine	12.05	12.80
	Pindolol	9.10	9.08
	Pirbuterol	7.17	6.42
	Prilocaine	9.14	8.72

Table 2 (*continued*)

Compound structure	Pharmaceutical name	$t_{m(plain)}$ [min]	$t_{m(CSA)}$ [min]
	Propafenone	10.27	13.60
	Salbutamol	9.83	8.39
	Sotalol	9.55	9.65
	Tocainide	8.18	7.07
	Trimipramine	9.09	14.18

$t_{m(plain)}$ = migration time in plain phosphate buffer.
$t_{m(CSA)}$ = migration time in cyclodextrin containing phosphate buffer.
Conditions as quoted in Table 1.

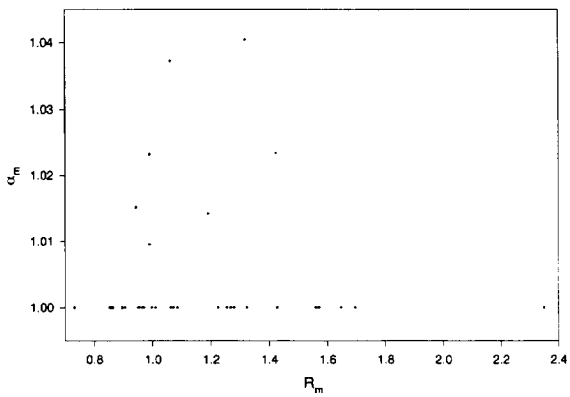


Fig. 3. Plot of separation factor α_m versus retardation factor R_m, for all compounds investigated.

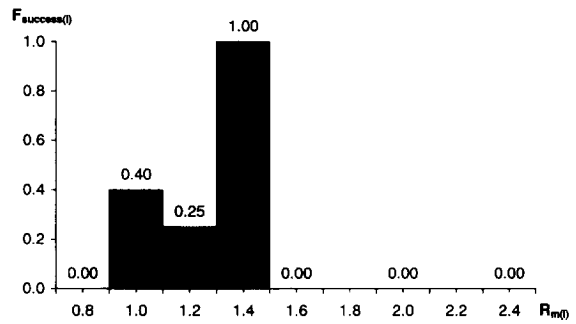


Fig. 4. Ratio of compounds separated and compounds not separated, in different R_m regions.

Fig. 4, displaying the ratio of analytes separated and analytes not separated into enantiomers, $F_{success(i)}$, for each slice (i) of R_m, see Eq. 6.

$$F_{success(i)} = \text{analytes}_{separated(i)} / \text{analytes}_{unseparated(i)} \quad (6)$$

This optimum can be tentatively explained by the relationship between c_{opt}, see Eq. 4, and the complex formation constants K_1 and K_2. Large complex formation constants (K) imply small optimum selector concentrations (c_{opt}). A strong retardation may have two different reasons, either a high ratio μ_u/μ_c or a high complex formation constant of the second eluted enantiomer (K_2). Whereas the first possibility would generally favour the separation, the second implies a location of the α_m maximum at a CSA concentration lower than the one applied in this experimental series. Further optimization experiments are on the way to decide on this question.

In summary, a total ratio of success ($F_{success}$) close to 1:4 looks encouraging in view of the simplicity of these experiments with β-cyclodextrin, and the possibility of further optimization. In the case of Atenolol, Bupivacaine and Pindolol, the triethanol ammonium ion in the background electrolyte may be decisive for the successful resolution of the enantiomers [22].

After all, one should be aware of the difficulty of comparison on the basis of either unified conditions on one hand, or individually optimized conditions on the other; both approaches have inherent drawbacks. An interpretation of complex formation constants (both, absolute values and ratio between both enantiomers), that will be possible after experiments at various cyclodextrin concentrations, will provide a better insight into the molecular recognition than that which is possible from the separation factor at one distinct concentration.

All data of this and other parts of the screening program are electronically stored in the factual molecular database Chirbase/CE, along with all literature data. Eventually, this database will provide an indispensable knowledge basis, similar to other parts of Chirbase on LC, SFC and GC [25,26], for selecting the most promising chiral selector and suitable experimental conditions.

Acknowledgments

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References

[1] K. Williams, E. Lee, Drugs, 30 (1985) 333.
[2] B. Koppenhoefer, R. Behnisch, U. Epperlein, H. Holzschuh, Perfum. Flavor., 19 (1994) 1.
[3] S.C. Stinson, Chem. Eng. News, Sept. 19 (1994) 38.
[4] B. Koppenhoefer, U. Epperlein, P. Piras, C. Roussel, Poster presented at the 17th Int. Symp. on Capillary Chromatography and Electrophoresis, Wintergreen (VA, USA), May 7-11, 1995
[5] J. Snopek, I. Jelinek, E. Smolkova-Keulemansova, J. Chromatogr., 438 (1988) 211.
[6] I. Jelinek, J. Snopek, E. Smolkova-Keulemansova, J. Chromatogr., 439 (1988) 386.
[7] A. Guttman, A. Paulus, A.S. Cohen, N. Grinberg, B.L. Karger, J. Chromatogr., 448 (1988) 41.
[8] S. Birnbaum, S. Nilsson, Anal. Chem., 64 (1992) 2872.
[9] H. Nishi, T. Fukuyama, M. Matsuo, S. Terabe, J. Microcolumn Sep., 1 (1989) 234.
[10] A. Dobashi, T. Ono, S. Hara, J. Yamaguchi, Anal. Chem., 61 (1989) 1984.
[11] S. Mayer, V. Schurig, J. High Resolut. Chromatogr., 15 (1992) 129.
[12] S. Li, D.K. Lloyd, Anal. Chem., 65 (1993) 3684.
[13] E. Gassmann, J.E. Kuo, R.N. Zare, Science 230 (1985) 813.
[14] S. Fanali, L. Ossicini, F. Foret, P. Bocek, J. Microcolumn Sep., 1 (1989) 190.
[15] B. Lin, Y. Ji, U. Epperlein, B. Koppenhoefer, Poster presented at HPCE '95, Wuerzburg (Germany), Jan 29 - Feb 2, 1995.
[16] B. Lin, Y. Ji, Y. Chen, U. Epperlein, B. Koppenhoefer, Chromatographia, 42 (1996) in press.
[17] D. Sybilska, J. Zukowski, in 'Chiral Separations by HPLC: Applications to Pharmaceutical Compounds', A.M. Krstulovic, ed., Ellis Horwood Limited, Chichester, 1989, p. 148.
[18] M. Heuermann, G. Blaschke, J. Chromatogr., 648 (1993) 267.
[19] S.A.C. Wren, R.C. Rowe, J. Chromatogr., 603 (1992) 235.
[20] S.A.C. Wren, J. Chromatogr., 636 (1993) 57.
[21] K. Otsuka, S. Terabe, J. Liq. Chromatogr., 16 (1993) 945.

[22] I. Bechet, P. Paques, M. Fillet, P. Hubert, J. Crommen, Electrophoresis Weinheim Ger., 15 (1994) 818.
[23] Y.Y. Rawjee, D. Staerk, G. Vigh, J. Chromatogr., 635 (1993) 291.
[24] Y.Y. Rawjee, R.L. Williams, L.A. Buckingham, G. Vigh, J. Chromatogr. A, 688 (1994) 273.
[25] B. Koppenhoefer, A. Nothdurft, J. Pierrot-Sanders, P. Piras, C. Popescu, C. Roussel, M. Stiebler, U. Trettin, Chirality, 5 (1993) 213.
[26] B. Koppenhoefer, R. Graf, H. Holzschuh, A. Nothdurft, U. Trettin, P. Piras, C. Roussel, J. Chromatogr. A, 666 (1994) 557.