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Rapid development of the enantiomeric separation of β-blockers by capillary electrophoresis using an experimental design approach

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Abstract

A rapid method for determining the separation conditions for chiral resolution of eleven β-blocking drug substances by capillary electrophoresis is described, using an experimental design approach. An acidic phosphate–triethanolamine buffer and an uncoated fused-silica capillary were used for all experiments. Several modified cyclodextrins were applied as chiral selectors: sulfobutyl ether β-cyclodextrin (SBE-βCD), dimethyl β-cyclodextrin (DM-βCD), carboxymethyl β-cyclodextrin (CM-βCD), and hydroxypropyl β-cyclodextrin (HP-βCD). Two different fractional factorial experimental designs were applied: (1) a design examining four factors at three levels (3^{4-2}) and (2) one examining three factors at two levels (2^{3-1}). The factors studied were: type of cyclodextrin, cyclodextrin concentration, pH of the background electrolyte and percentage of organic modifier. Enough resolution for the separation of the enantiomers and even for their quantification was reached. The same scheme is proposed when a fast chiral separation method needs to be developed for other drug families.

Keywords: Enantiomer separation; Experimental design; β-Blockers

1. Introduction

In the last two decades, particularly in the pharmaceutical industry, special emphasis has been placed on the synthesis of enantiomerically pure compounds. This emphasis can be attributed in large part to an increased awareness that pharmacological and toxicological differences can exist between enantiomers [1]. Therefore, chiral considerations are now an integral part of drug research and development and of the regulatory process [2].

Rapid and efficient separation of stereoisomers continues to be a challenge for the separation scientist. A universal technique for the separation of optical active compounds does not exist. Currently, chromatographic methods have been widely used in the routine analysis of chiral compounds [3,4]. In the past few years capillary electrophoresis (CE) has become a powerful alternative due to the inherent speed and efficiency of the technique as well as due to its capability of rapid optimization [5].

The chiral β-blockers have at least one chiral center in their side chain and most of them are

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marketed as racemic mixtures except *S*-timolol and *S*-penbutolol. Differences in activity among the enantiomers of the β-blockers are well known [6]. An example is propranolol that has an *S*-enantiomer that is 100 times more potent as a β-blocking agent than the *R* enantiomer. It is also known that *R*- and *S*-sotalol have similar antiarrhythmic activities [7] but only the *R*-enantiomer exhibits the β-blocking activity [8].

For the enantiomeric separation of β-blockers, chromatographic methods have been commonly used. A review of these methods has been written by Vandenbosch et al. [9]. Supercritical fluid chromatography (SFC) [10–11] and micellar electrokinetic chromatography (MEKC) were also reported in the analysis of β-blockers [12].

For enantiomeric separations by CE, cyclodextrins (CD) are the simplest chiral selectors that can be used because they are inexpensive, provide a fast equilibration of the CD–solute complex and have a high efficiency and a good peak symmetry. The hydroxyl groups of the CD (primary and secondary) can easily be modified using chemical reactions to obtain CDs with different properties, thus extending the application range.

In CE, the structure of the analyte determines the choice of the cyclodextrin to be used. However, among structurally similar compounds, the slight differences in structure between two substances may prevent enantiomeric resolution. Therefore, in the method development often several cyclodextrins need to be tested for each analyte. Optimization of the chiral separation conditions usually involves the use of the classical trial and error method. Several cyclodextrin types and concentrations, background electrolyte (BGE) compositions and pHs are tested, and generally one factor at a time is changed. This kind of procedure involves a large number of time consuming experiments.

In order to predict the influence of some factors on separations, several studies have been performed during recent years. Wren and Rowe [12,13] have developed a theoretical model relating mobility to the CD concentration. They showed that an optimal CD concentration exists for a particular chiral separation. After the selection of the proper CD type and pH, the CD optimal concentration can be predicted. However, this requires that the equilibrium constant of each enantiomer complex formation should be known or that it should be calculated experimentally (including the associated acid–base equilibrium of the analyte, pK_a values). Vigh et al. [14] developed a model for mobility, chiral selectivity and peak resolution that takes into account the simultaneous effects of both pH and CD concentration on the separation of the enantiomers of weak electrolytes. These models recognise three different types of interactions of the analyte when CD are used for chiral separations and give an idea about the pH range that can be used for the separation. However, a final tuning of the conditions must be done, either using sequential optimization or experimental design.

In industrial applications, it often happens that a certain separation will be applied only once or just a few times and it is not possible to spend sufficient time and experimental work on the separation optimisation. Furthermore, it requires the intervention of an expert and so when, as it often happens in industry, many separations have to be developed, this places a too large burden on this expert. This situation occurs, for instance, when a large number of compounds must be checked for enantiomeric purity, such as products from combinatorial chemistry and/or from drug development. In these specific cases, the optimal results are not of interest but the region, where the results are sufficiently good, is.

Some attempts were already made in order to simplify the method development of chiral separation. Guttman et al. [15,16], suggested the step by step cyclodextrin array chiral analysis to make a selection of the proper CD and pH in few experiments. Roos et al. [17], tested the applicability of the Elphodextrine kit for the separation of some chiral drugs. Lurie et al. [18] proposed the use of mixtures of neutral and anionic cyclodextrins for the rapid separation of basic compounds. Fillet et al. [19,20] suggested some rules for the use of sequential optimization conditions that may reduce the time needed by a considerable extent. Vigh et al. [14] proposed (according to the predictions of their CHARM model and after the promising CD is found) the use of only two series of measurements (one at low and one at high pH at fixed CD concentration) before the optimization of the CD concentration and electroosmotic flow (EOF) value. These approaches have been shown to be very useful in many contexts but they still need much expertise.

Strategies that do allow a feasible, although not

necessarily optimal, separation to be found with relatively little knowledge and experimental work should be a useful addition. In this work, a rapid method for determining the separation conditions for the chiral resolution of 11 β-blockers by capillary electrophoresis is described using an experimental design approach. It should be understood that the objective of the experimental design here is not to find an optimal separation, but to explore the experimental domain in such a way that one has a good chance of finding acceptable separation conditions by only executing a limited number of experiments.

2. Experimental

2.1. Apparatus and reagents

A fully automated capillary electrophoresis instrument CE ultra, TermoQuest Co. (San Jose, CA, USA) equipped with a fast scanning UV–VIS detector was used for all measurements. An uncoated fused-silica capillary 50 μm I.D., 43.3 cm total length (37.1 cm length to the detector cell) was utilized. Phosphoric acid, triethanolamine, and hydroxyethylcellulose were provided by MERCK (Darmstadt, Germany), carboxymethyl-β-cyclodextrin (CM-βCD) and trimethyl-β-cyclodextrin (TMβCD) by Cyclolab R&D Lab. (Budapest, Hungary), sulfobutylether-β-cyclodextrin (SBE-βCD) by BioScience Innovations (Lawrence, USA), hydroxypropyl-β-cyclodextrin (HP-βCD) and dimethyl-β-cyclodextrin (DM-βCD) by Beckman Instruments (Fullerton, CA, USA). The racemic β-blockers, drug substances metoprolol, oxoprenolol, and alprenolol, were purchased from Novartis (Basel, Switzerland), acebutolol from Rhone-Poulenc (Vitry Sur Seine, France), labetalol from GlaxoWellcome (Hertfordshire, UK), propranolol, atenolol, bunitrolol, pindolol, sotalol, and toliprolol were gifts from diverse sources.

2.2. Methodology

The BGE phosphate–triethanolamine buffer was prepared by titration of a 100 m*M* phosphoric acid solution with triethanolamine until reaching the required pH. Preconditioning of the capillary was done daily with 0.1 *M* NaOH, water, and buffer respectively for 5 min each at 100 p.s.i. The temperature was set at 15°C. Between runs a pre-wash of 3 min at 100 p.s.i with BGE followed by a 1 min capillary filling with the selected cyclodextrin in the run buffer was always done. Hydrodynamic injection of the sample for 10 s at 0.8 p.s.i was performed. An electric field of 25 kV was applied for the separation. Detection at 214 nm was performed.

Table 1
Description of the factors and levels for the 3^{4-2} fractional factorial design

Factors	Levels		
	−1	0	1
CD type	CM	DM	HP
Concentration of CD (m*M*)	5	15	30
pH	2.5	4	5.5
MeOH (%)	0	15	30

The β-blockers were screened using two different fractional factorial experimental designs: (1) a design for four factors at three levels (3^{4-2}) and (2) a design for three factors at two levels (2^{3-1}). The factors examined in the first design were (1) type of CD; (2) CD concentration; (3) pH of the background electrolyte (BGE); and (4) percentage of organic modifier, methanol (MeOH), and in the second design the three last factors were examined (Tables 1–4). As response functions, the resolution between the *R*- and *S*-enantiomers and the analysis time (migration time of the last peak) were determined.

3. Results and discussion

While the effects of some of the operating parameters on the separation of enantiomers by CE, such as the type of CD [21–25], the type and concentration of organic modifier [13,23,26–29] and the type and

Table 2
Description of the factors and levels of the 2^{3-1} fractional factorial design

Factors	Levels	
	−1	1
Concentration of CD (m*M*)	5	30
pH	2.5	5.5
MeOH (%)	0	30

Table 3
The 3^{4-2} fractional factorial design and the measured response results (resolution) for propranolol and atenolol

Experiment number	Factors				Response (R_S[a])	
	CD type	Concentration of CD	pH	MeOH (%)	PR	AT
1	−1	0	0	1	1.41	0.68
2	−1	1	−1	0	1.83	0.00
3	−1	−1	1	−1	0.00	0.72
4	0	1	0	−1	0.67	0.52
5	0	0	1	0	0.00	0.00
6	0	−1	−1	1	0.43	0.00
7	1	0	−1	−1	0.91	0.00
8	1	−1	0	0	0.93	0.00
9	1	1	1	1	0.00	0.00

[a] R_S = Resolution, values <0.3 were considered to be zero.
PR = propranolol, AT = atenolol.

concentration of the buffer, are difficult to predict, the effects of other parameters such as the pH [30–33] and the concentration of the chiral selector [12] can be predicted. For instance, if the pK_a of the analyte is known it is possible to predict the pH range where the ionic form is present.

The parameters that were considered to have the most influence in the chiral separation of the compounds studied by CE were the type of cyclodextrin, its concentration, the pH of the BGE and the percentage of organic modifier. Therefore, these parameters were examined in the experimental designs (Tables 1–4). However, the separation also depends on other factors such as the electroosmotic flow [34], the ionic strength and the co-ion of the background electrolyte [35–38], the presence of additives (triethanolamine, cellulose, urea) [39–41] and the temperature [42]. These secondary parameters, which were not examined in the experimental design, were kept constant (see methodology (Section 2.2)).

Table 4
The 2^{3-1} fractional factorial design applied to screen the β-blockers

Experiment number	Factors		
	Concentration of CD	pH	MeOH (%)
1	1	1	1
2	−1	1	−1
3	1	−1	−1
4	−1	−1	1

In CE, a good equilibration of the capillary is necessary before each experiment. The optimization of the cleaning and equilibration procedure between runs was observed to be very important to obtain reproducible data. A pre-wash step of 3 min at 100 p.s.i with BGE was found to be efficient. In this way approximately 60 capillary column volumes were applied to ensure the equilibrium of the capillary wall to a new set of conditions.

β-blockers are basic drugs due to the presence of the secondary amino group in their structure (Fig. 1). They have relatively high pK_a values (9.2–9.8). According to Vigh et al. [14] for weak bases, ionoselective interaction (i.e. the dissociated form complexes with the CD) can take place at low pH. Therefore, to have the drugs in a charged form, low pH values (2.5 and 5.5) were selected to be tested in the experimental design. When a normal polarity was applied the ions migrated towards the cathode.

The modified CD were preferred to the natural ones because they present several advantages in terms of solubility, increased cavity depth and the occurrence of additional groups that can interact and stabilise the CD–drug complex. The chiral selectors available for this purpose were HP-βCD, DM-βCD, CM-βCD and SBE-βCD. The last two selectors are charged CD, which, in addition to a better solubility, possess an intrinsic electrophoretic mobility that allows the separation of both charged and neutral

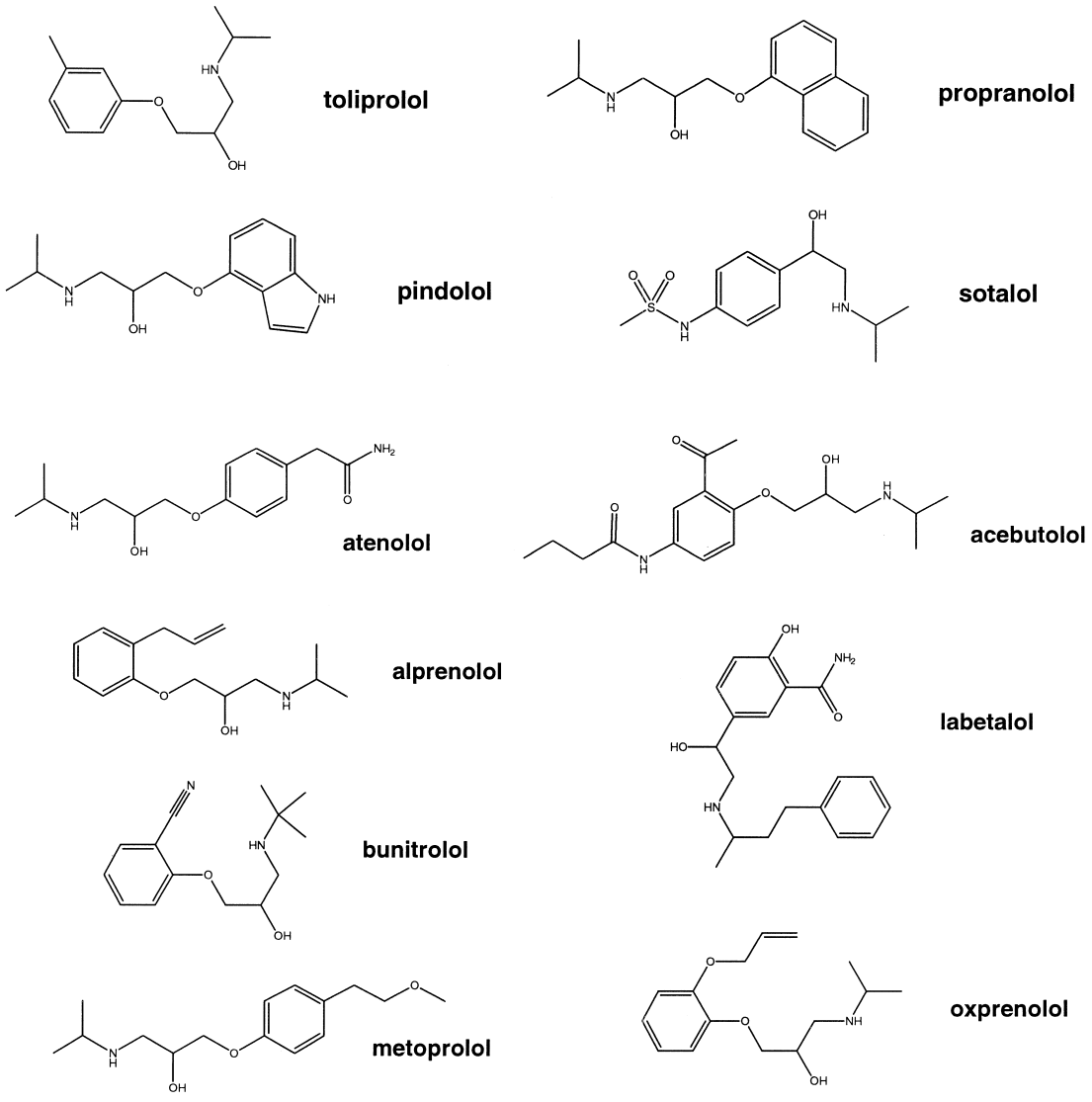

Fig. 1. Molecular structures of the β-blockers.

chiral compounds [43–49]. The enantiomeric separation is based on the formation of diastereomeric complexes due to the drug inclusion in the CD cavity, in addition to the electrostatic interactions of the analyte with the functional groups of the CD upper rim that can also occur [50].

In the first experiments, the influence of four parameters, each at three levels, on the resolution of propranolol and atenolol was studied. Nine experiments were carried out in duplicate. The 3^{4-2} fractional factorial design and the responses (resolution) for these compounds are shown in Table 3. The type of chiral selector (CMβ-CD, DM-βCD and HP-βCD) was included as a factor in the experimental design. Due to their similar solubilities the same concentration ranges could be applied for them.

For each factor three effects can be estimated, namely for the intervals between the levels [1,0],

[0,−1] and [1,−1]. Only two of those three effects are independent. Therefore, the following effects were calculated (Eqs. (1) and (2)).

$$E_{x[1,0]} = \frac{\sum Y(1)}{N/3} - \frac{\sum Y(0)}{N/3} \quad (1)$$

$$E_{x[0,-1]} = \frac{\sum Y(0)}{N/3} - \frac{\sum Y(-1)}{N/3} \quad (2)$$

in which ΣY(1), ΣY(0), and ΣY(−1) represent the sum of the responses where the factor x is at level 1, 0 and −1 respectively and N is the number of design experiments. The aim of this evaluation is to select the experimental conditions that lead to a good separation, i.e. an acceptable resolution in the examined domain.

The effect of each parameter on the peak resolution was calculated and is shown graphically in Fig. 2. Evaluation of the results indicates that for propranolol two factors caused an important effect (CD type and pH). For the type of CD used CM-βCD presented the best separation while, on the other hand, the highest pH examined caused a worse separation than the lower ones. In the case of atenolol three effects were found to be rather important, namely caused by the type of CD, the pH and the percentage of MeOH. Again CM-βCD was found to give the best resolution of the CD examined. The resolution also seemed to increase when the pH was higher than 2.5 and when no organic modifier was added.

For these two β-blockers, a clear tendency of CM-βCD to be a more effective selector compared to DM-βCD and HP-βCD was observed. Therefore, a more economical 2^{3-1} fractional factorial design was applied to screen all β-blockers using CM-βCD. In this two-level design, the set-up contained four experiments (executed in duplicate) (Table 4). The resolution values obtained are shown in Table 5. The effects of each parameter were calculated according to Eq. (3)

$$E_{x[-1,+1]} = \frac{\sum Y(-1)}{N/2} - \frac{\sum Y(+1)}{N/2} \quad (3)$$

The effects of the factors do not show a general tendency but behave in a particular way for each compound (Table 6). An acceptable set of conditions could be predicted from the effects observed for each compound. For instance, for propranolol using the factor level −1, two factors (CD concentration and pH) lead to an increase in resolution, while at the same level the percentage of MeOH makes the separation worse. Therefore, when using the level −1 for CD concentration and pH and the level +1 for the percentage of MeOH, the best resolution of the different factor levels that were examined is expected. If the predicted set of conditions was not

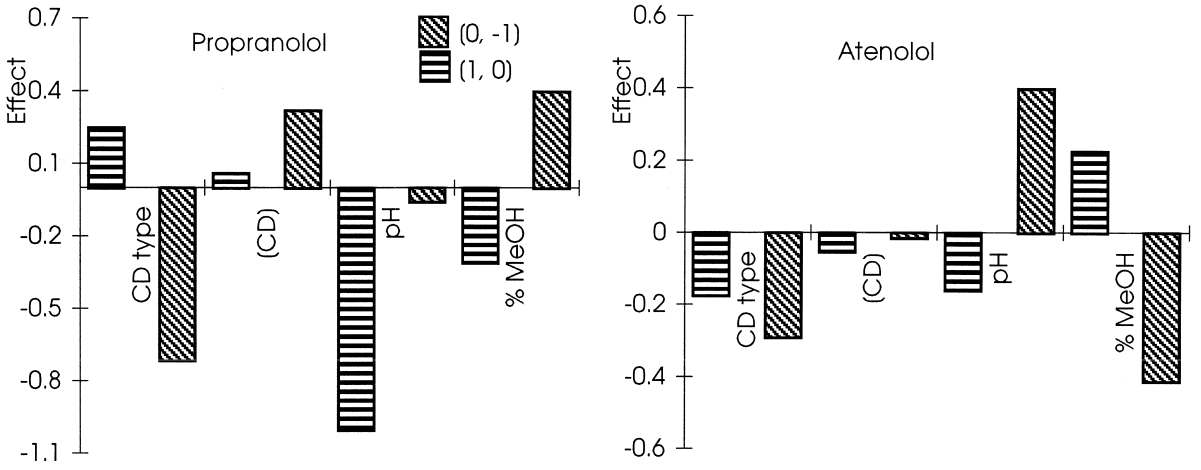

Fig. 2. Calculated main effects from the 3^{4-2} fractional factorial design applied to screen propranolol and atenolol.

Table 5
Resolution values obtained for the eleven β-blockers using the 2^{3-1} fractional factorial design and CM-βCD as the chiral selector

Experiment number	PR	AT	AC	AL	LA	BU	ME	OX	PI	SO	TO
1	0.00	0.62	0.59	0.30	0.00	**1.27**	0.00	**1.85**	1.10	**1.56**	0.00
2	0.00	0.88	0.37	1.22	0.00	0.52	0.00	0.00	**1.79**	0.52	**0.69**
3	1.58	0.39	0.59	1.47	**2.07**	1.07	0.00	1.20	1.22	0.81	0.00
4	**3.56**	0.00	0.00	**2.41**	1.68	0.46	**0.62**	0.46	0.70	0.59	0.56
Add[a]	–	**1.24**	0.64	–	–	–	–	–	–	–	–

[a] An additional experiment was done at condition (1,1,−1).
PR=propanolol, AT=atenolol, AC=acebutolol, AL=alprenolol, LA=labetalol, BU=bunitrolol, ME=metoprolol, OX=oxprenolol, PI=pindolol, SO=sotalol, TO=toliprolol.

in the original design, an additional experiment was performed at these best conditions. An increase in the resolution values for atenolol and acebutol was observed when the predicted conditions were used (Table 5). The conclusion shown earlier from the 3^{4-2} design for the more important effects on the resolution of propanolol and atenolol was confirmed.

In order to select another more selective CD for the compounds which had resolution values (R_S) less than 1.0 using CM-βCD, the 3^{4-2} design was repeated. This was the case for acebutolol, metoprolol and toliprolol, having resolution values of 0.64, 0.62 and 0.69 respectively, when CM-βCD was used (Table 5).

The CD tested in the new three-level design were HP-βCD, DM-βCD and the charged SBE-βCD. The measured responses can be seen in Table 7. The effect of the factors was calculated using Eqs. (1) and (2). For the three substances a clear increase in resolution was observed when SBE-βCD was used as a chiral selector. Therefore, the 2^{3-1} fractional factorial design was then applied to screen the remaining drugs, acebutolol, metoprolol and toliprolol, using SBE-βCD as a chiral selector. The factors were varied as in Table 2, except that the CD concentration levels used were 5 and 10 m*M*. The responses obtained are shown in Table 8. The calculated effects of the factors and the experiment number that yielded the highest result can be seen in Table 9. For the three screened compounds the resolution was the best when 10 m*M* SBE-βCD, pH 5.5 and 30% methanol were used.

Table 7
Response (resolution) for acebutolol, metoprolol and toliprolol using the 3^{4-2} fractional factorial design[a]

Experiment number	AC	ME	TO
1	0.00	0.57	0.65
2	0.00	0.00	0.95
3	0.00	0.00	0.31
4	0.00	0.00	0.00
5	0.00	0.00	0.00
6	0.00	0.00	0.00
7	0.60[b]	0.31[b]	1.35[b]
8	0.30	0.45	0.60
9	7.40	1.10[b]	1.80

[a] R_S values <0.3 were considered to be zero.
[b] Reverse polarity was applied (−25 kV).

Table 6
Calculated effects and predicted experiments (best CE conditions) for the 11 β-blockers using the 2^{3-1} fractional factorial design and CM-βCD as the chiral selector

Factor	PR	AT	AC	AL	LA	BU	ME	OX	PI	SO	TO
Concentration of CD (m*M*)	0.99	−0.06	−0.40	0.93	−0.20	−0.68	0.31	−1.30	0.08	−0.64	0.62
pH	2.57	−0.56	−0.18	1.18	1.88	−0.13	0.31	−0.10	−0.49	−0.34	−0.07
MeOH (%)	−0.99	0.32	0.19	−0.01	0.20	−0.07	−0.31	−0.55	0.60	−0.41	0.07
Predicted experiment[a]	4	Add[b]	Add	4	3	1	4	1	2	1	2

[a] Predicted experiment according the calculated effects ($E_{x[-1,1]}$).
[b] Additional experiment at conditions (1, 1, −1).

Table 8
Resolution values obtained using the 2^{3-1} fractional factorial design and SBE-βCD as the chiral selector

Experiment number	AC	ME[a]	TO[a]
1	**11.95**	**1.21**	**2.92**
2	0.50	0.62	0.00
3	0.31	0.54	0.00
4	0.00	0.00	0.00

[a] Reverse polarity was used (−25 kV).

Table 9
Calculated effects and predicted experiments (best CE conditions) for the 2^{3-1} fractional factorial design using SBE-βCD as the chiral selector

Factor	AC	ME	TO
Concentration of CD (m*M*)	5.88	0.56	1.46
pH	6.07	0.64	1.46
MeOH (%)	5.57	0.02	1.46
Predicted experiment[a]	1	1	1

[a] Predicted experiment according to the calculated effects ($E_{x[1,-1]}$).

In this two-level design better separation was obtained than in the three-level one because the SBE-βCD concentration range was selected particularly for the CD that was used, while in the three-level design an equal range was applied for all CD types.

The three-level design is clearly useful for the selection of the best CD and to make a first guess for good conditions of the remaining factors. Two representative compounds of a family can be used to make this selection. Only nine experiments are needed to be carried out to know which of the three CD tested is the most promising for a certain group of compounds with similar structures, such as the β-blockers. Then, the use of a two-level design allows the rest of the group to be screened in a more economical way because only four experiments need to be performed. In addition, occasionally better conditions for the separation can be predicted with the calculation of the effects. This results in a practical scheme for the rapid and economical development of the chiral separation.

It was confirmed experimentally that the charged CD are more effective chiral selectors than the neutral ones for the β-blockers. The modified cyclodextrin CM-βCD leads to a good resolution in eight cases, while SBE-βCD was preferred in three cases. The best separation conditions and the corresponding analysis times found for the β-blockers using the 2^{3-1} fractional factorial design are shown in Table 10. The resolution values reached are very promising (for three compounds $1.20 < R_S < 1.50$ and for eight compounds $R_S > 1.50$). Only four experiments with each selector were necessary to obtain these results. In the case of labetalol, which has two chiral centers,

Table 10
Best CE separation conditions for the β-blockers found using the 2^{3-1} fractional factorial design[a]

Drug name	Number of chiral carbons	CD type	Concentration of CD (m*M*)	pH	MeOH (%)	Resolution	Analysis time (min)
Propranolol	1	CM	5	2.5	30	3.56	19.70
Atenolol	1	CM	30	5.5	0	1.24	9.00
Acebutolol	1	SBE	10	5.5	30	11.95	11.93
Alprenolol	1	CM	5	2.5	30	2.41	14.55
Labetalol	2	CM	30	2.5	0	2.07[b]	11.76
Bunitrolol	1	CM	30	5.5	30	1.27/1.40[c]	9.26/9.75
Metoprolol	1	SBE	10	5.5	30	1.21[d]	14.71
Oxoprenolol	1	CM	30	5.5	30	1.85	9.27
Pindolol	1	CM	5	5.5	0	1.79/1.88[c]	6.91/6.48
Sotalol	1	CM	30	5.5	30	1.56	12.38
Toliprolol	1	SBE	10	5.5	30	2.92[d]	23.85

[a] BGE = 100 m*M* phosphate–triethanolamine; drug concentration = 20 μg/m; 25 kV; 15°C; fused-silica capillary 50 μm, 40 cm (33.5 cm to the window).
[b] Two of the four enantiomers were separated.
[c] Addition of 0.005% hydroxypropylcellulose.
[d] Reverse polarity (−25 kV).

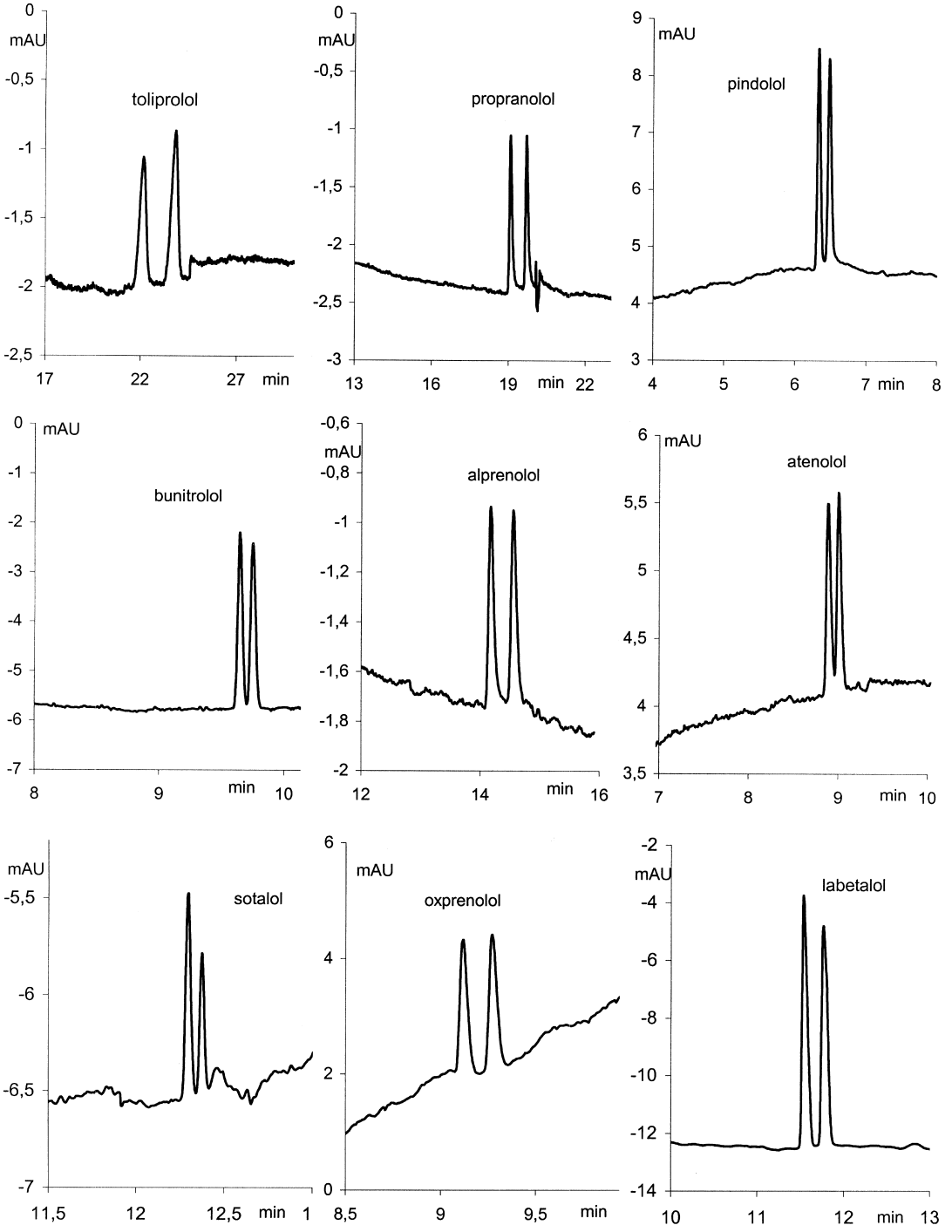

Fig. 3. Electropherograms of the enantiomeric separation of the β-blockers under the optimal conditions predicted as the best. The separation conditions for each compound are specified in Table 10.

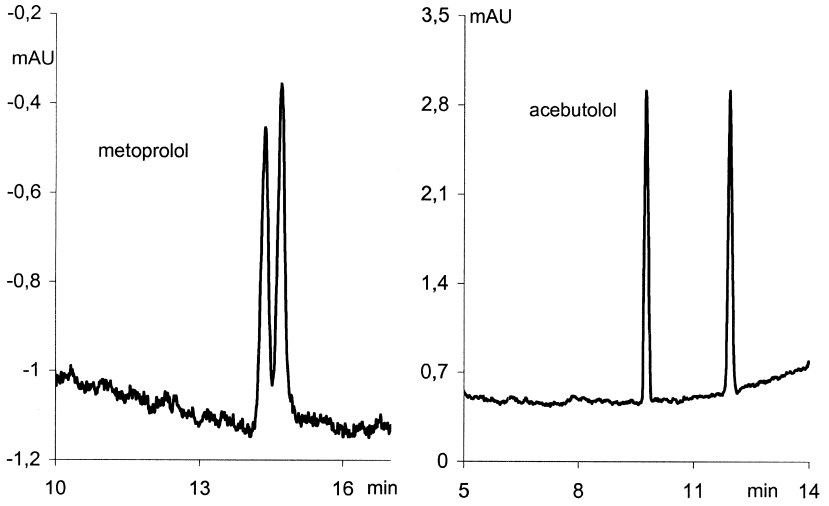

Fig. 3. (*continued*)

only two of the four enantiomers were separated using this scheme.

The modified CM-βCD is a weak acid ($pK_a = 4.5$). The carboxylic group can be charged or uncharged depending on the pH of the BGE. It was observed that for lipophilic compounds, such as propranolol, labetalol and alprenolol (log *P* values of 2.98, 2.4 and 3.1 respectively [51]), a good resolution was obtained when the CM-βCD was used at low pH (2.5), where this CD is in the protonated form (Table 10). A possible explanation is that the chiral selector behaves as a quasi stationary phase (at this pH) and hydrogen bonds to stabilise the complex with these lipophilic analytes can be formed. For more hydrophilic drugs (i.e. atenolol, sotalol, log *P* of 0.16 and −0.65, respectively), pH 5.5 was more efficient for the separation. At this pH the CD is charged due to the dissociation of the carboxylic groups and migrates with its own mobility. An additional ion-pair interaction is suggested for a stable complex formation [43,49].

In the case of acebutolol, metoprolol and toliprolol, the SBE-βCD was the best selector. In the last two cases (metoprolol and toliprolol) reverse polarity was applied. SBE-βCD is a negatively charged molecule (strong acid) over the whole pH range that has its own mobility in the opposite direction of the analytes. Therefore, the drug–CD complexes formed migrate toward the anode providing a resolution improvement in these cases. As is described in Wren and Rowe's model [12], the greater the mobility difference between the free and complexed analyte, the better the resolution. Therefore, charged CD are expected to give better resolutions than the neutral CD for enantiomers that bear a charge of opposite sign.

The electropherograms for the chiral separation of the analyzed compounds are shown in Fig. 3. The use of a longer capillary can increase the resolution (Fig. 4) but unacceptable migration times of more than 35 min are necessary for the analysis.

3.1. Influence of the drug structure

Compounds which contain two rings in their structure (Fig. 1) have the highest resolution values (pindolol, propranolol and labetalol, log *P* of 1.75, 2.98, and 2.41, respectively) using CM-βCD as a chiral selector (Table 5). Alprenolol (log $P = 2.81$) also has a high resolution value, which may be due to the large carbon chain in the *ortho*-position that leads to a similar steric interaction as that of compounds with an additional ring. It is also possible that due to this characteristic they fit better in the hydrophobic cavity of the cyclodextrin because the two rings impart high lipophilicity and, therefore, possibly a higher affinity for the CD. The high

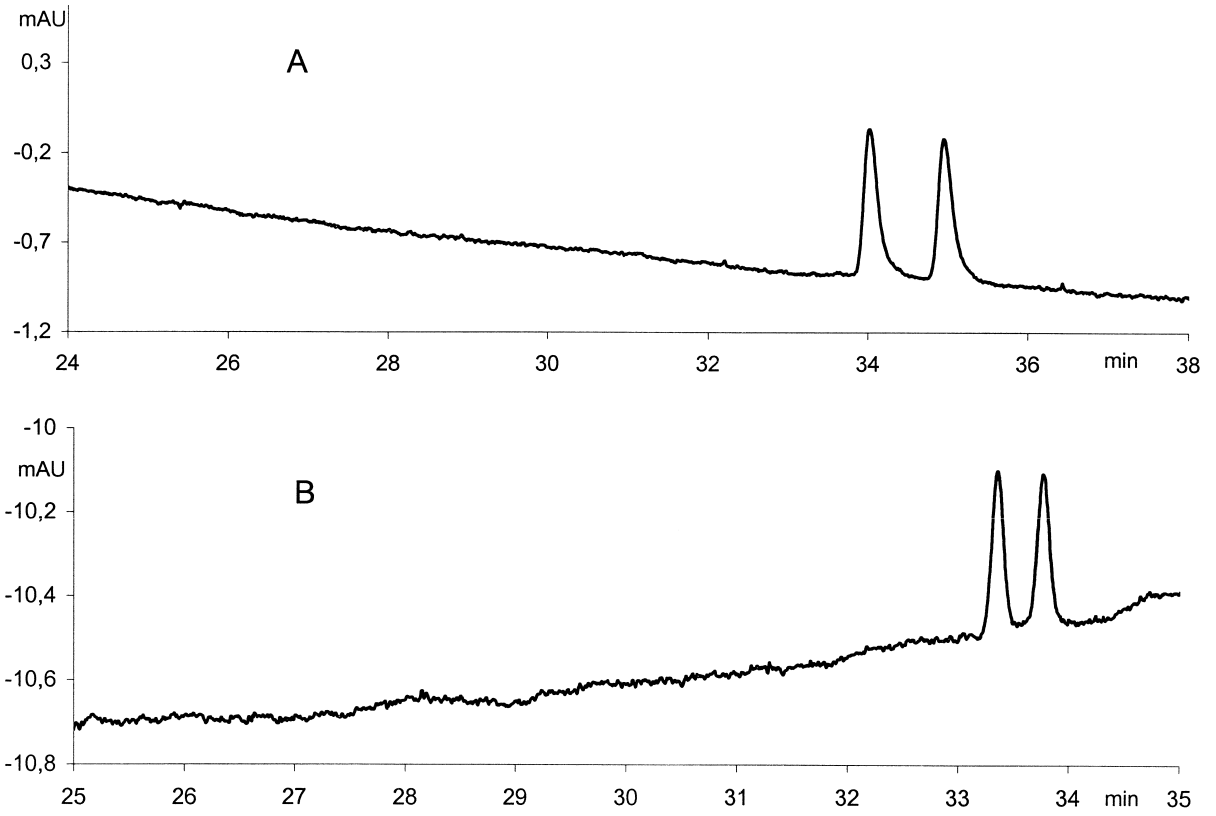

Fig. 4. Electropherograms of the chiral separation of two β-blockers using a longer capillary: (A) alprenolol ($R_S = 3.1$) and (B) bunitrolol ($R_S = 2.9$). Fused-silica capillary 75 μm I.D., 60.4 cm total length (54.4 cm length to the detector).

affinity was observed for the low CD concentration that was used (even methanol was used to decrease the drug–CD interaction) for the separation of all these analytes, except for labetalol.

Compounds which have a large chain substituent in the *para*-position tend to have the lowest resolution (acebutolol, metoprolol) when CM-βCD is used (Table 5). This can be due to the steric hindrance produced by that chain that does not allow the molecule to interact with the outer groups of the cyclodextrin, which can stabilise the complex. However, the same compounds were resolved when SBE-βCD was the selector (Table 8).

3.2. Influence of dynamic modifiers

Dynamic modifiers (which are fixed to the capillary wall in a reversible way) such as triethanolamine and/or hydroxyethylcellulose (HEC) can help to improve the resolution because they can suppress the electroosmotic flow (EOF). For chiral separations, the EOF is considered to be an additional parameter to be optimized for the separation [34]. Triethanolamine was added to the buffer solution in all the experiments. It can suppress and even reverse the EOF at a pH around 3. The use of this modifier in CE was introduced by Fillet et al. [19] to reduce the EOF effects.

At a pH higher than 5, in order to maintain a very small EOF value, the addition of HEC to the buffer was tested. A significant decrease in the current at pH 5.5 was obtained. The addition of HEC to the BGE helps to avoid the Joule heat generation but an improvement in the peak shape and/or resolution was observed in only a few cases, such as for bunitrolol and pindolol (Fig. 5).

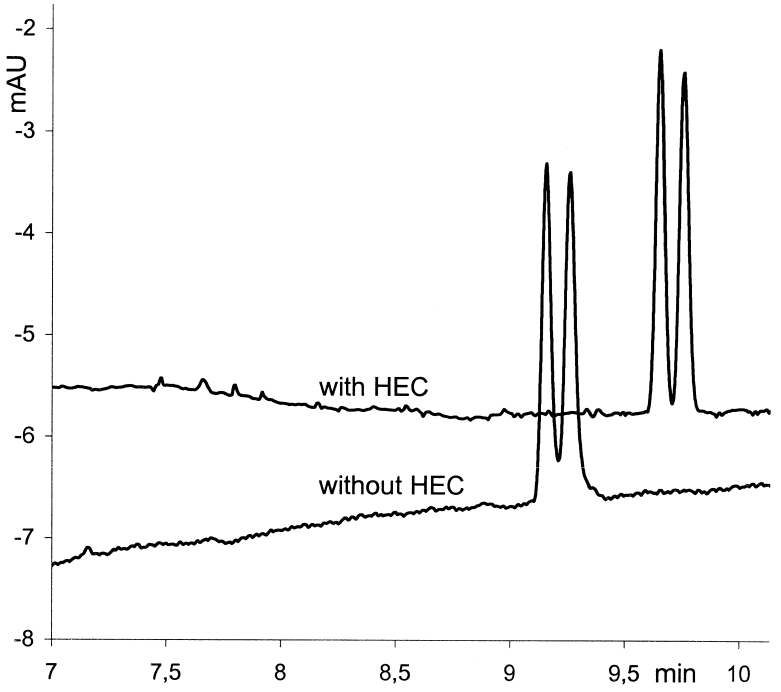

Fig. 5. Electropherograms of the enantiomeric separation of bunitrolol with or without the addition of hydroxyethylcellulose as a dynamic modifier.

4. Conclusion

A practical scheme for the fast enantiomeric separation of the β-blocking agents was developed using an experimental design approach. A 3^{4-2} fractional factorial design applied to some representative compounds of a drug family was an effective way for the selection of the most suitable CD. A 2^{3-1} fractional factorial design proved to be an economical set of experiments to provide enough resolution of the enantiomers. The selection of the factors to be fixed and of those to be varied is a crucial step for the success of this experimental approach. Experimental design, well applied, is cost saving, only four experiments had to be carried out for each compound if a good selection of factors had been made. Fractional factorial designs were employed to reduce the number of experiments without needed recourse to an expert. The results shows that this aim was achieved.

Charged CDs were used as effective chiral selectors for the enantiomeric separation of this family of basic compounds. CM-βCD leads to a good resolution value in eight cases while SBE-βCD is preferred in three cases. The resolution of the β-blockers can be increased if a longer capillary is used but the analysis time increases unacceptably. The same experimental scheme will be tested in future for the development of enantiomeric separations of other basic drugs. One possible improvement that we will investigate is if the addition of a central point to the two-level designs can help to find good conditions occurring at intermediate levels. This would have advantages from an experimental design point of view and would give an indication as to whether better results can be achieved at intermediate values of the factors.

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