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Enantiomeric purity determination of propranolol by cyclodextrin-modified capillary electrophoresis

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Abstract

A capillary electrophoretic method for the enantiomeric purity determination of either enantiomer of propranolol was developed using cyclodextrins as chiral additives and uncoated fused-silica capillaries thermostated at 15°C. The effect of the type and concentration of cyclodextrin added to a triethanolamine–phosphate buffer (pH 3.0) on chiral resolution and migration times was studied. The propranolol enantiomers could be separated with all cyclodextrins tested (β-cyclodextrin and seven of its derivatives), except dimethyl-β-cyclodextrin. A particularly high resolution value of 4.4 was obtained for propranolol enantiomers with a buffer containing 10 m*M* carboxymethyl-β-cyclodextrin. This buffer was selected for testing the enantiomeric purity of propranolol, making it possible to reach detection limits of less than 0.1% for the minor enantiomer. The *R* enantiomer of propranolol (second migrating) could be quantified at the 0.5% level with good precision (intra-day R.S.D. = 1.7%) in samples of the *S* enantiomer (first migrating), while the limit of quantification of the latter, when present as an impurity in the *R* enantiomer, was 0.1%. The method also gave good results in terms of linearity and accuracy.

1. Introduction

Most often the enantiomers of chiral drugs have different pharmacological and toxicological properties and therefore the quantitative enantiomeric composition of these drugs should be determined [1–3]. The separation and determination of enantiomers are required for enantiomeric purity testing, chiral stability testing of pharmaceutical formulations (absence of racemization of drug enantiomers) and in pharmacokinetic and clinical studies.

Capillary electrophoresis (CE) using cyclodextrins as chiral selectors has proved to be very useful in chiral analysis, owing to its high separation efficiency and enantioselectivity [4–6]. However, only few papers have considered so far quantitative aspects of chiral CE [7–9].

Propranolol (cf., Fig. 1) is a β-adrenergic blocking agent, i.e., a competitive inhibitor of the effects of catecholamines at β-adrenergic receptor sites. It is widely used in therapeutics for its antihypertensive, antiangorous and antiarrhythmic properties. Pharmacological studies

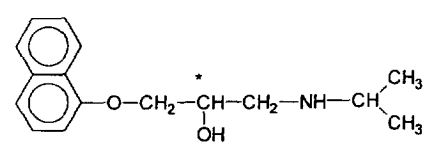

Fig. 1. Structure of propranolol.

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have shown that the *S*-(−) enantiomer of propranolol is about 100 times more active than the *R*-(+) enantiomer [10].

Native and derivatized cyclodextrins [11–17] have often been used in free solution capillary zone electrophoresis (CZE) for the enantioseparation of propranolol. The resolution of propranolol enantiomers could be obtained by adding β-cyclodextrin or one of its derivatives to phosphate buffers of low pH (2.4–3.1) [11–16]. With carboxymethyl-β-cyclodextrin, ionizable at higher pH, chiral resolution for propranolol was improved by increasing the buffer pH up to 5.8 [14]. Propranolol was also enantioseparated by CZE by use of a running buffer containing hydroxypropyl-β-cyclodextrin, an uncharged derivative, and a hydrophilic polymeric additive at pH 7.0 [17].

In addition to cyclodextrins, cellulase was found to be a good chiral selector for the CZE enantiomeric separation of β-blockers, including propranolol [18]. Micellar electrokinetic capillary chromatography (MEKC) was also recently applied to the resolution of propranolol enantiomers, using either sodium dodecyl sulfate with cyclodextrins [19] or a chiral surfactant, such as N-dodecoxycarbonylvaline [20].

In this work, a CZE method for the enantiomeric purity determination of propranolol was developed. β-Cyclodextrin and seven of its derivatives were tested as chiral additives. The effect of the type and concentration of cyclodextrin on the resolution and migration behaviour of propranolol enantiomers was studied, using a triethanolamine phosphate buffer (pH 3.0) and uncoated fused-silica capillaries thermostated at 15°C. Optimum conditions with respect to chiral resolution were selected for testing the enantiomeric purity of propranolol. This enantioselective CE method has been validated and the results of this validation are given.

2. Experimental

2.1. Apparatus

All experiments were performed on a Model ^{3D}CE system (Hewlett-Packard, Palo Alto, CA, USA) equipped with a diode-array detector, an automatic injector, an autosampler and a temperature control system (15–60°C, ±0.1°C). An HP Vectra 486/66XM computer was used for instrument control and data handling. The pH of the buffers was adjusted by means of a Delta 345 pH meter (Mettler, Halstead, UK).

2.2. Chemicals and reagents

Phosphoric acid (85%) and triethanolamine were of analytical-reagent grade from Merck (Darmstadt, Germany). Water was of Milli-Q quality (Millipore, Bedford, MA, USA). β-Cyclodextrin (βCD) and heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (TMCD) were purchased from Sigma (St. Louis, MO, USA), heptakis (2,6-di-O-methyl)-β-cyclodextrin (DMCD) and hydroxypropyl-β-cyclodextrin (HPCD) from Janssen Chimica (Geel, Belgium) and carboxymethyl-β-cyclodextrin (CMCD), carboxyethyl-β-cyclodextrin (CECD), succinyl-β-cyclodextrin (SCD) and methyl-β-cyclodextrin (MCD) from Cyclolab (Budapest, Hungary). Propranolol hydrochloride racemate was kindly supplied by SMB (Brussels, Belgium). (*R*)-(+) and (*S*)-(−)-propranolol hydrochlorides were purchased from Sigma. All compounds were used without further purification.

2.3. Electrophoretic technique

Separations were carried out with uncoated fused-silica capillaries (48.5 cm × 50 μm I.D., 40 cm to the detector). Before use, the capillary was washed successively with basic solutions (i.e., 1 *M* NaOH followed by 0.1 *M* NaOH), water and separation buffer. The latter consisted of 0.1 *M* phosphoric acid adjusted to pH 3 with triethanolamine. At the beginning of each working day, the capillary was washed with separation buffer for 10 min, and after each sample injection the capillary was washed with water for 2 min and with buffer for 3 min.

The applied voltage was 25 kV and UV detection was performed at 210 nm. Injections were made using the hydrodynamic mode (injection pressure 5 kPa) for 2 s (resolution studies) or 30 s (enantiomeric purity testing). The capillary was

thermostated at 15°C. The standard solutions were prepared by dissolving salts of racemic propranolol at a concentration of 50 μg/ml and salts of pure enantiomers at a concentration of 25 μg/ml.

The resolution (R_s) was calculated according to the standard expression based on peak width at half-height [21].

3. Results and discussion

3.1. Buffer composition

All enantiomeric separations reported were performed with buffers made of 100 m*M* phosphoric acid adjusted to pH 3.0 with triethanolamine and containing β-cyclodextrin or one of its derivatives [15]. Under these conditions, analyte interactions with the capillary wall were minimized and the electroosmotic flow was reversed, with a low, fairly constant mobility value of $-4 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1}, which gave rise to highly reproducible migration times. The use of triethanolamine, a co-ion of low mobility, also resulted in good peak symmetry for cationic analytes such as propranolol, low currents (40–60 μA) and high efficiency (ca. 100 000 theoretical plates per capillary in the low concentration range). At the pH used, propranolol was fully ionized whilst all cyclodextrins tested were principally in uncharged form.

3.2. Influence of the type and concentration of cyclodextrin

β-Cyclodextrin (βCD) and seven of its derivatives, methyl-β-cyclodextrin (MCD), heptakis-(2,6-di-O-methyl)-β-cyclodextrin (DMCD), heptakis (2,3,6-tri-O-methyl)-β-cyclodextrin (TMCD), hydroxypropyl-β-cyclodextrin (HPCD), carboxymethyl-β-cyclodextrin (CMCD), carboxyethyl-β-cyclodextrin (CECD) and succinyl-β-cyclodextrin (SCD), were tested as chiral selectors for the enantiomeric separation of propranolol, injected as racemate.

Table 1 shows the influence of the nature and the concentration of the eight cyclodextrins on the enantioseparation of propranolol. A dash indicates that no resolution was observed between the enantiomers ($R_s < 0.5$) and a resolution value lower than 0.7 means that the enantiomers were partially resolved but not sufficiently to permit a precise determination of R_s.

Table 1 clearly shows that large differences in chiral resolution are generally obtained with the different cyclodextrins at a given concentration. The propranolol enantiomers can be completely resolved with all β-cyclodextrin derivatives ex-

Table 1
Effect of cyclodextrin type and concentration on resolution of propranolol enantiomers

CD	Cyclodextrin concentration (m*M*)							
	1	2	5	10	15	20	30	50
DMCD	–[a]	–	–	–	–	–	–	–
HPCD	0.8	1.5	2.2	2.4	2.3	2.0	1.8	1.4
TMCD	–	–	0.7	1.3	1.6	1.8	2.0	2.1
βCD	–	<0.7	1.0	1.1	1.0	/[b]	/	/
CMCD	1.0	1.6	3.0	4.4	/	/	/	/
CECD	<0.7	1.0	1.4	1.4	1.6	1.5	1.3	/
MCD	0.9	1.4	1.9	1.5	1.4	1.2	0.9	/
SCD	<0.7	0.7	1.3	1.4	1.5	1.5	1.2	/

Buffer, 100 m*M* phosphoric acid adjusted to pH 3 with triethanolamine containing β-cyclodextrin or one of its derivatives. Injection mode, hydrodynamic, 5 kPa, 2 s. Other conditions as described under Experimental.

[a] – No detectable resolution.

[b] /, Not determined (migration times longer than 50 min.).

cept DMCD, which gives no resolution in the concentration range studied. With the native β-CD, only partial resolution was obtained.

Table 2 shows the influence of the type and concentration of cyclodextrin on the migration times of propranolol. When chiral resolution is obtained, the migration times, given in Table 2, are those of the first enantiomer of propranolol. An increase in migration time with increasing cyclodextrin concentration was observed in all cases, even when the increase in buffer viscosity was taken into account, indicating that the propranolol enantiomers have a more or less pronounced tendency to interact with all cyclodextrins, including DMCD.

As can be seen from Table 1, for each cyclodextrin there is an optimum concentration at which chiral resolution reaches a maximum value [12,15]. Three of the β-cyclodextrin derivatives (TMCD, HPCD and CMCD) were found to be particularly suitable, since they gave resolution values higher than 2. A maximum resolution value of 4.4 was obtained for propranolol enantiomers with a buffer containing 10 m*M* CMCD. A typical electropherogram obtained under these conditions is presented in Fig. 2. The buffer giving the highest resolution was selected for testing the enantiomeric purity of propranolol.

3.3. Validation of the method developed for the enantiomeric purity testing of propranolol

Several performance criteria were studied, including selectivity, linearity of detector response, limits of detection and quantification for the minor enantiomer, accuracy and reproducibility of peak area measurements.

Selectivity

The homogeneity of the peaks of propranolol enantiomers (absence of interferences from the sample matrix) was tested by use of the diode-array detector. Each enantiomer was then injected alone and no trace of the other enantiomer was detectable, which demonstrated the enantiomeric purity of the propranolol enantiomers tested. The first migrating peak was identified as the *S* enantiomer of propranolol (the more active enantiomer) and the second migrating peak as the *R* enantiomer (the less active).

Linearity

Two calibration graphs were constructed in order to demonstrate the linearity of the detector response (cf., Table 3): the first in the range 0.1–10% of the *S* enantiomer and the second in the range 0.5–10% of the *R* enantiomer in

Table 2
Effect of cyclodextrin type and concentration on migration times of propranolol enantiomers

CD	Cyclodextrin concentration (m*M*)							
	1	2	5	10	20	30	50	
DMCD	9.6	10.7	13.1	16.0	18.2	19.5	22.0	29.9
HPCD	11.1	12.5	15.4	19.0	21.4	23.2	26.1	31.2
TMCD	9.1	9.4	9.9	10.5	11.4	12.4	14.3	17.7
βCD	9.9	10.6	12.9	15.5	17.3	/[a]	/	/
CMCD	10.9	13.1	17.3	21.3	/	/	/	/
CECD	9.9	12.5	15.6	19.1	25.7	28.9	34.2	/
MCD	10.9	11.6	14.7	18.3	18.7	19.8	22.2	/
SCD	9.1	9.9	11.8	14.4	16.6	18.1	21.9	/

Buffer, 100 m*M* phosphoric acid adjusted to pH 3 with triethanolamine containing β-cyclodextrin or one of its derivatives. Injection mode, hydrodynamic, 5 kPa, 2 s. Other conditions as described under Experimental.
[a] Not determined (migration times longer than 50 min).

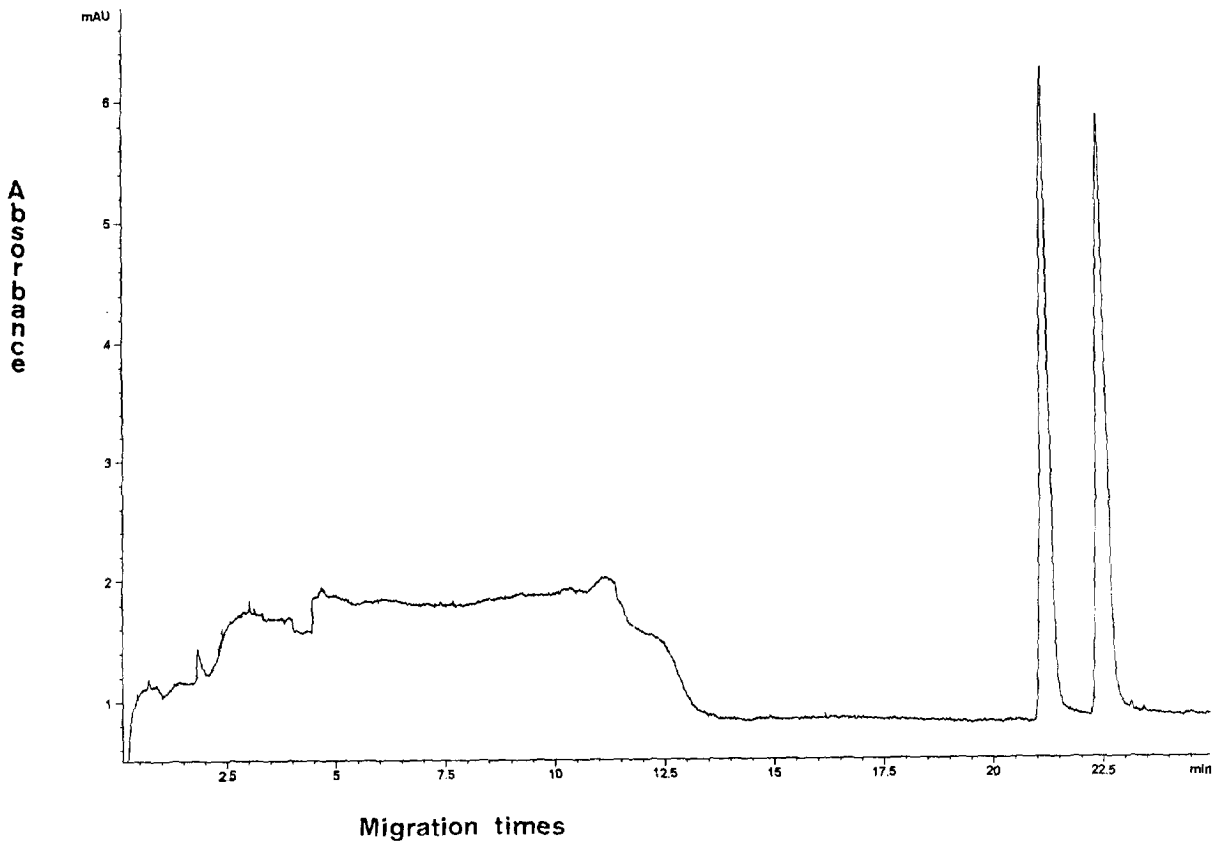

Fig. 2. CE separation of propranolol enantiomers. Buffer: 10 m*M* carboxymethyl-β-cyclodextrin in 100 m*M* phosphoric acid adjusted to pH 3 with triethanolamine. Hydrodynamic injection: 5 kPa, 2 s. Samples: 50 μg/ml solution of racemic propranolol in diluted buffer. Other conditions as under Experimental.

samples of their stereoisomer, each calibration point ($n = 5$) being injected in triplicate. Linear regression analysis, plotting the analyte peak area (y) versus the percentage of impurity (x), gave the following equations:

S enantiomer: $y = 9.25x + 0.44$ (± 0.86)

R enantiomer: $y = 7.61x - 0.05$ (± 0.39)

The adequate linearity of the calibration graphs is demonstrated by the determination coefficients ($r^2 > 0.999$) obtained for the regression lines.

Limits of detection and quantification

Limits of detection (LOD) and quantification (LOQ), corresponding to signal-to-noise ratios of 3 and 10, respectively, were calculated from linear regression analysis made by plotting the analyte peak height versus the percentage of impurity.

LODs of 0.03% and 0.06% and LOQs of 0.10% and 0.22% were obtained for the *S* and *R* enantiomers, respectively (cf., Table 3). These limits are particularly low for the *S* enantiomer, which migrates first. Typical electropherograms of propranolol enantiomers containing low levels

Table 3
Linearity, limits of detection and quantification

Parameter	*S* enantiomer	*R* enantiomer
Calibration range (%)	0.1–10	0.5–10
Calibration points	5	5
Slope ± S.D.	9.25 ± 0.06	7.62 ± 0.03
Intercept ± S.D.	0.44 ± 0.30	−0.05 ± 0.14
S.D. of residuals	0.86	0.39
Coefficient of determination (r^2)	0.9995	0.9998
Comparison of the intercept to zero (Student's test):		
t calculated	1.46	0.36
t from the table	2.16	2.16
Existence of a significant slope (Fisher's test):		
F_1 calculated	25604	77678
F_1 from the table	4.67	4.67
Limit of detection (%)	0.03	0.06
Limit of quantification (%)	0.10	0.22

Table 4
Reproducibility of peak areas

Spiking level of the minor enantiomer (%)	*n*	R.S.D. (%)	
		S enantiomer	*R* enantiomer
Intra-day precision			
10	6	0.5	1.5
1	6	2.8	1.9
0.5	6	ND[a]	1.7
0.1	6	3.4	–[b]
Inter-day precision			
10	3	0.9	1.8
1	3	3.2	5.2
0.5	3	ND[a]	8.5
0.1	3	20.4	–[b]

[a] Not determined.
[b] Below to the limit of quantification.

of their stereoisomers are presented in Fig. 3. Limits of detection and quantification for the *R* enantiomer could probably be improved by the injection of smaller volumes of more concentrated samples.

Accuracy

The accuracy of the method at the 0.1% and 0.5% levels for the *S* and *R* enantiomers, respectively, in samples of their stereoisomers were also determined in terms of recovery. The following results were obtained: *S* enantiomer, 96.77 ± 8.13%; and *R* enantiomer, 97.77 ± 3.74%. As the theoretical value of 100% was included in the confidence interval, the test procedure could be considered accurate over the range studied.

Reproducibility

The relative standard deviations (R.S.D.s) for peak area measurements are given in Table 4.

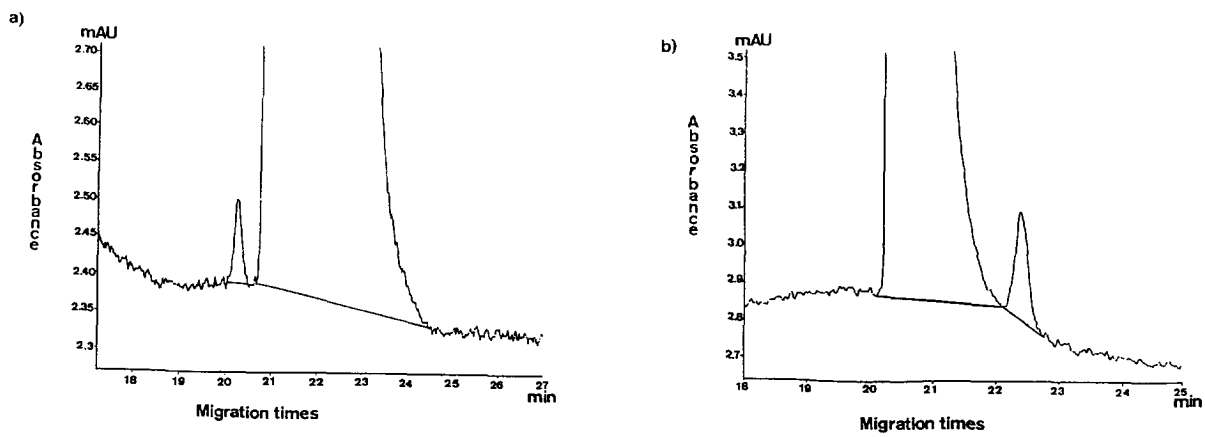

Fig. 3. Typical electropherograms of propranolol enantiomers containing low levels of their stereoisomers. (a) 0.1% of *S* enantiomer in *R* enantiomer; (b) 0.5% of *R* enantiomer in *S* enantiomer. Hydrodynamic injection: 5 kPa, 30 s. Samples: 25 μg/ml solutions of propranolol enantiomers in diluted buffer. Other conditions as in Fig. 2.

The intra-day precision was evaluated at three percentages for each enantiomer. The inter-day precision was studied on four different days. The intra-day R.S.D.s are 1.7% for 0.5% of the *R* enantiomer and 3.4% for 0.1% of the *S* enantiomer in samples of their stereoisomers. The inter-day R.S.D.s at the LOQ are relatively high but still acceptable for such low levels of the minor enantiomer.

Stability of sample solution

Solutions of both enantiomers were stored for 3 months at 22°C and analysed again by the chirally selective CE method. No racemization of the enantiomers had occurred, indicating a sample solution shelf-life of at least 3 months.

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