Chiral Analysis of Metoprolol and Its By-Products by Capillary Electrophoresis

Zoltán Juvancz,[1] Karin E. Markides,[2] László Jicsinszky[3]
[1]*VITUKI Public Limited Company, Institute for Water Pollution Control H-1095, Kvassay J. út 1, Budapest, Hungary*
[2]*Uppsala University, Department of Analytical Chemistry, Box 531, S-751 21, Uppsala, Sweden*
[3]*Cyclolab Ltd., H-1525, Dombovári út 5, Budapest, P.O.B. 435, Hungary*

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Abstract: The enantiomers of metoprolol, a frequently used β-blocker, have different physiological effects, which makes their chiral separation necessary. The separation of the main product from its by-products and their chiral separation were also aims of this study. Capillary electrophoresis (CE) with cyclodextrin (CD) additives, providing high efficiency and selectivity, was applied for this goal. Separation of the main product from 5 by-products as well as their chiral separation was realized. Fifteen CDs (native α, β, γ, and derivatives) were evaluated. Carboxymethylated α-CD (R_s 3.0), and phosphated α-CD (R_s 2.7) were the best for the chiral separation of metoprolol while carboxymethylated α-CD, phosphated α-CD, and phosphated γ-CD were appropriate for the enantioseparation of by-products and for their separation from the main product. The roles of the type and concentration of the chiral selectors, of the pH, and of the organic additives in the buffers were studied. The stacking effect of phosphated α-CD was investigated in detail. J Micro Sep 11: 716–722, 1999

Key words: *chiral CE; cyclodextrins; metoprolol; by-products*

INTRODUCTION

Metoprolol, 1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-2-propanol is a well known β_1-blocking agent. It is used for treatment of hypertension and angina pectoris. Most commercialized products contain the racemate of metoprolol, in spite of the fact that the two enantiomers have different physiological effects [1–3]. This difference requires stereoselective production and analysis of metoprolol. Some gas chromatography (GC) [4,5], supercritical fluid chromatography (SFC) [6], liquid chromatography (LC) [7], and capillary electrophoresis (CE) methods [3,8–13] have already been developed for the chiral separation of metoprolol. Our aims, however, were not only the chiral separation of the main product but also the separation of 5 by-products from metoprolol as well as their chiral separation. Cyclodextrins (CDs) were chosen as chiral selectors, because of their ability to separate positional isomers [3]. Fifteen different types of CDs were evaluated to fulfill the set goals. We mainly concentrated our study on anionic CDs, because of their proven high selectivity [3,8,13] and because their selectivity can be different toward analytes in neutral and charged states [14]. Compromises had to be made between the chiral separation of metoprolol and its separation from the by-products.

EXPERIMENTAL

Chemicals. CYCLOLAB Ltd. (Budapest, Hungary) produced all the CDs summarized in Table I. Chemicals for preparation of the 100 mM buffer solutions (H_3PO_4, NaH_2PO_4, Na_2HPO_4, $Na_2B_4O_7$, and $B(OH)_3$) were from Fluka (Buchs, Switzerland) and deionized water was obtained from a Milli-Q unit (Millipore, Millford, MA, USA). Hässle (Mölndal, Sweden) donated metoprolol and by-products (Figure 1).

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Correspondence to: Z. Juvancz
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Table I. *Characterization of CDs used*

Name of CDs	Abbreviation of CD	Structure of substituents	Degree of substitution	Position of substituents
α-Cyclodextrin	α-CD	—	—	—
Carboxymethylated α-cyclodextrin	α-CMCD	—CH_2COOH	2–3	2, 6
Carboxyethylated α-cyclodextrin	α-CECD	—C_2H_5COOH	2–3	2, 6
α-Cyclodextrin phosphate	α-PhoCD	—H_2PO_3	6–12	2, 6
β-Cyclodextrin	β-CD	—	—	—
Methyl β-cyclodextrin	RAMEB[a]	—CH_3	13–14	2, 3, 6
Dimethylated β-cyclodextrin	DIMEB[b]	—CH_3	14	2, 6
Trimethylated β-cyclodextrin	TRIMEB[c]	—CH_3	21	2, 3, 6
Carboxymethylated β-cyclodextrin	β-CMCD	—CH_2COOH	2–3	2, 6
Carboxyethylated β-cyclodextrin	β-CECD	—C_2H_5COOH	2–3	2, 6
β-Cyclodextrin phosphate	β-PhoCD	—H_2PO_3	6–12	2, 6
γ-Cyclodextrin	γ-CD	—	—	—
Carboxymethylated γ-cyclodextrin	γ-CMCD	—CH_2COOH	2–3	2, 6
Carboxyethylated γ-cyclodextrin	γ-CECD	—C_2H_5COOH	2–3	2, 6
γ-Cyclodextrin phosphate	γ-PhoCD	—H_2PO_3	6–12	2, 6

[a]Randomly methylated.
[b]Regularly (2,6) methylated.
[c]Permethylated.

Instrumentation. A P/ACE 2100 capillary electrophoresis system (Beckman, Fullerton, CA, USA) was used with the ultraviolet (UV) detector set at 214 nm. The analyses were done on 50 μm i.d. × 37 cm (effective length 30 cm) untreated fused silica tubing (Polymicro Technologies, Phoenix, AZ, USA) at 15°C. Hydrodynamic injections were done with 2 s injection time. Sample concentrations were 100 μg/mL in the running buffer. The applied voltage was adjusted to keep the current under 90 μA.

RESULTS AND DISCUSSION

A preliminary screen was performed for racemic metoprolol on the 15 CDs to select the more promising chiral agents and conditions for further studies (Table II). A 10 mM concentration of CD (except for α-PhoCD, 6.7 mM) was applied at three pH values.

The measurements at pH 2.4 were made with carboxyl substituted CDs only, because only these CDs are neutral at this pH value, while they are ionized at pH 4.9. Separations at pH 2.4 had, however, only limited success. Only β-CMCD showed a resolution value (R_s) of 0.7 for metoprolol.

All CSs used were tried at pH 4.9 and promising results were obtained. Under these conditions both metoprolol and the anionic CDs are charged. α-CMCD, α-CECD, α-PhoCD, RAMEB, β-CMCD, β-PhoCD, γ-CMCD, and γ-PhoCD showed measurable enantioselectivity. Under these conditions α-PhoCD showed the highest R_s value of 1.4.

At pH 8.9, metoprolol is partly neutral while the anionic CDs are negatively charged. Under these conditions α-CMCD, α-PhoCD, β-CMCD, β-PhoCD, and γ-CMCD showed chiral recognition features. α-PhoCD showed the best result (R_s value of 1.1).

In the next step, chiral separations of metoprolol were improved using selected CDs. An R_s value of 3.0 could be achieved using 20 mM α-CMCD at pH 6 (Figure 2). This concentration was the maximum for the concentration vs. resolution curve at this pH. On the other hand, at pH 4.9, 15 mM was the optimum α-CMCD concentration with a corresponding R_s value of 1.7. The slope of the concentration vs. resolution curve at pH 4.9 was more flat than with pH 6.0. It is also worth noting that a concentration of α-CMCD as low as 2 mM resulted in measurable resolutions (i.e., 0.6 and 0.5 R_s at pH 6.0 and 4.9, respectively). Under pH 3.5 and above pH 7.8 the chiral recognition feature of α-CMCD strongly decreased. Adding methanol as an organic modifier also caused severe loss of resolution.

α-PhoCD is also an appropriate chiral additive toward metoprolol, with first migration of the (*S*) isomer. The best resolution was R_s 2.7 within 13-min using 15 mM α-PhoCD at pH 7.8. In Figure 3, it can

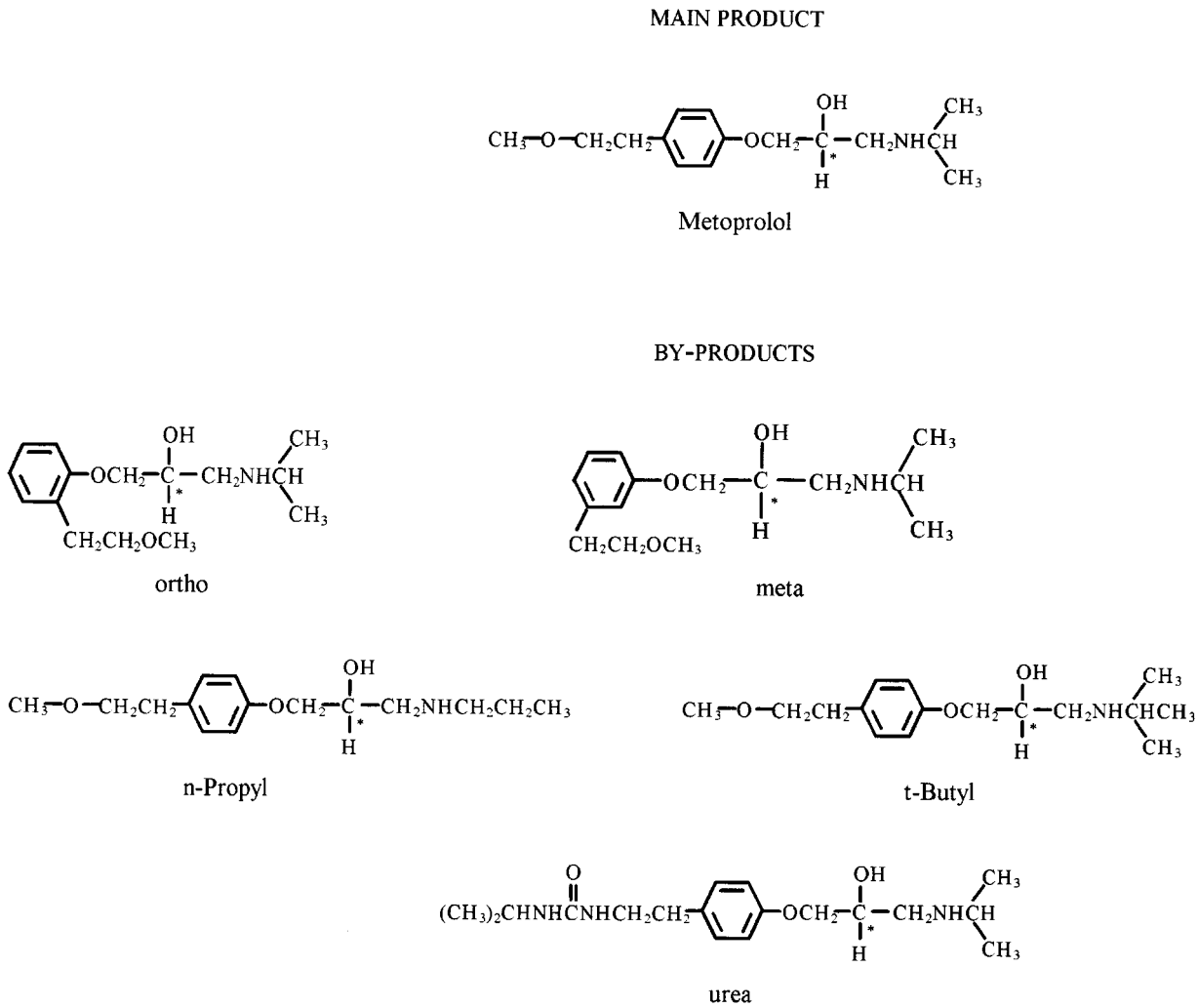


Figure 1. *Structures and abbreviations of metoprolol and its by-products.*

be seen that a resolution above 1.5 could be achieved in a broad pH interval from 4.9 to 8.9 using different chiral selector quantities. Despite a general practice in CE analysis of using β-blockers [8,12], the pH 2–3 range was not appropriate for the separation of metoprolol enantiomers using phosphated CDs. At these pH values, an α-PhoCD layer deposition was built up on the column wall [13]. This layer stacked the metoprolol, destroying the resolution and stopping the migration. The stacking effect was also the reason that metoprolol was not detected at pH 4.9 using 15 mM α-PhoCD. The stacking effect could, however, be decreased by decreasing the concentration of α-PhoCD. A concentration of 6.7 mM α-PhoCD at pH 4.9 thus produced an R_s value of 1.4. Addition of an organic modifier is another way to reduce the described stacking effect. The use of 15 mM α-PhoCD + 5% methanol at pH 4.9 resulted in an R_s value of 1.6. Increasing the pH is also a good way to reduce or even eliminate the stacking effect. The stacking effect continuously diminishes with increasing pH. As shown in Figure 3, the use of 15 mM α-PhoCD at pH 7.8 was an appropriate concentration.

Use of 5% methanol as a modifier improved the resolution of metoprolol at pH 4.9, because of a decrease of the stacking effect. On the other hand, the methanol modifier caused a resolution decrease at pH 7.8. For example, 15 mM α-PhoCD showed an R_s value of 2.7, which decreased to 2.3 when 5% methanol was added. At higher pH values, namely 8.3 and 8.9, addition of 5% methanol, however, also improved the resolution values of metoprolol compared to buffers without methanol. The maximum of the concentration vs. resolution curve at these pH values was probably at lower concentration values

Table II. *Chiral separations of metoprolol achieved in the first screen using 10 mM CDs*

Abbreviation of CD	Resolution		
	pH 2.4	pH 4.9	pH 8.9
α-CD	—	< 0.5	—
α-CMCD	< 0.5	1.1	0.9
α-CECD	< 0.5	0.8	< 0.5
α-PhoCD[b]	—	1.4	1.1
β-CD	—	< 0.5	—
RAMEB	—	0.8	—
DIMEB	—	< 0.5	—
TRIMEB	—	< 0.5	—
β-CMCD	0.7	0.9	0.8
β-CECD	< 0.5	< 0.5	< 0.5
β-PhoCD	n.d.	1.2	0.8
γ-CD	—	< 0.5	—
γ-CMCD	< 0.5	0.8	0.7
γ-CECD	< 0.5	< 0.5	< 0.5
γ-PhoCD	n.d.	1.2	< 0.5

[a]—, not measured.
[b]6.7 mM of selective agent was applied.
[c] < 0.5, no resolution was observed.
[d]n.d., not detected.

than 15 mM α-PhoCD. This is also supported by the R_s value of 2.3, which was achieved at pH 8.3 using 10 mM α-PhoCD (Figure 4) instead of R_s 2.0 for 15 mM α-PhoCD. To speed up the analysis time, a compromise was made between resolution and migration time. Use of 15 mM α-PhoCD + 5% methanol at pH 8.3 results in an R_s value of 2.3 in less than 7 min (Figure 5) . The profile of the concentration vs. resolution curve in the present situation was more complex than what was reported by Wren and Rowe [8]. Here, resolutions were influenced not only by complexation of enantiomers in the mobile phase but in the stacked layer too. α-PhoCD produced the most significant stacking effect of the CDs tested, but the two other phosphated CDs also showed similar behavior but to a lesser extent. β-PhoCD also had good chiral recognition features, producing an R_s value of 1.5 at pH 7.8 using 10 mM β-PhoCD.

γ-PhoCD can only be applied to a limited extent for the chiral separation of metoprolol. The use of 15 mM γ-PhoCD + 5% methanol resulted in an R_s value of 1.4 at pH 4.9. On the other hand, 5 mM γ-PhoCD produced an R_s value of 0.9, which is the same as that with 15 mM γ-PhoCD without a methanol modifier. At higher pH values the resolution of metoprolol diminished with use of γ-PhoCD. It is important to note that the (*R*) isomer migrated first, which is the opposite of the migration order on α-PhoCD. The migration order reversal suggests that the inclusion phenomenon is not crucial for the chiral recognition of metoprolol using CDs.

Metoprolol could be separated from its by-products, and most of the by-products were also enantiomerically separated, with α-CMCD. Metoprolol enantiomers were excellently separated in less than 15 min from the *ortho* and *meta* by-products using 15 mM α-CMCD at pH 4.9 (Figure 6). The best chiral separation was measured between the enantiomers of the *ortho* isomers (R_s value of 3.2).

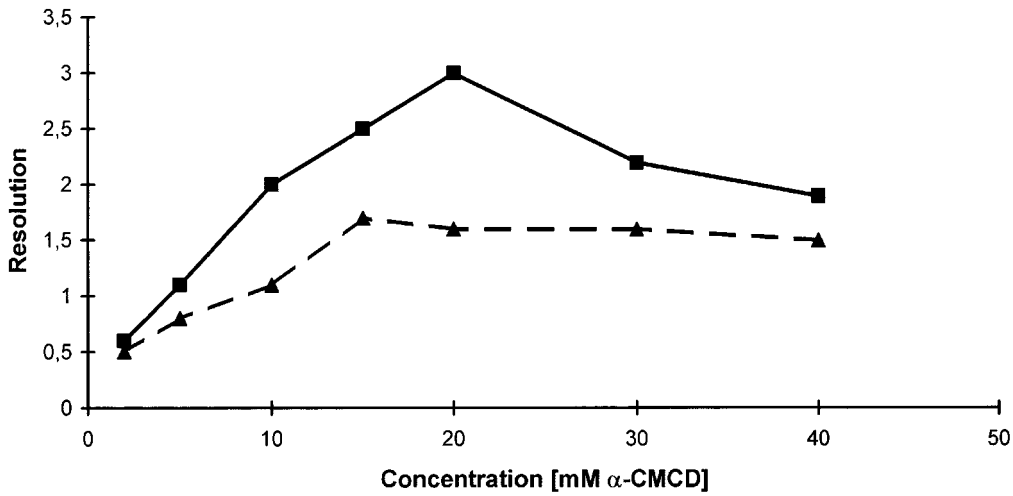


Figure 2. *R_s values of metoprolol as a functions of α-CMCD concentration. Conditions: (30 cm effective length) × 0.05 mm, i.d. open tubular fused silica column, UV at 214 nm; ▲, pH 4.9; ■, pH 6.0.*

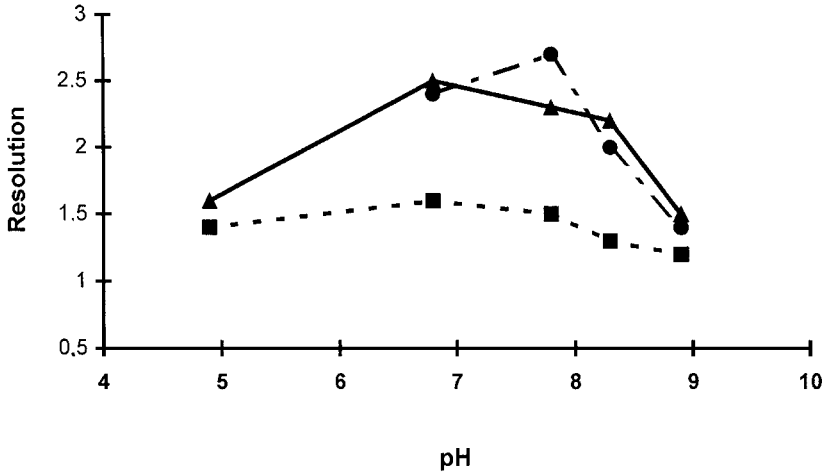
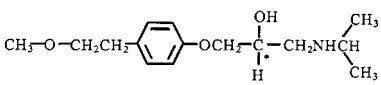


Figure 3. *R_s values of metoprolol as a function of pH using α-PhoCD. Conditions: column as the legend to Figure 2. ●, 15 mM α-PohoCD; ▲, 15 mM α-PhoCD + 5% methanol; ■, 6.7 mM α-PhoCD.*

Metoprolol itself shows only a slightly better resolution (R_s value of 1.7), than *meta* (R_s value of 1.6). Under the same conditions, the *t*-butyl by-product had an R_s value of 1.3, but elution is very close to the second peak of metoprolol. The *n*-propyl by-product had an R_s value of only 0.8. In addition, they partly overlapped with the second peak of the *t*-butyl by-product. The urea by-product showed no chiral selectivity and migrated before the *ortho* by-product. Adding 5% methanol, the enantiomer selectivity of the system decreased drastically, and the peaks of metoprolol partly overlapped with the *meta* isomers. Using 6.7 mM α-CMCD at pH 6.0, meto-



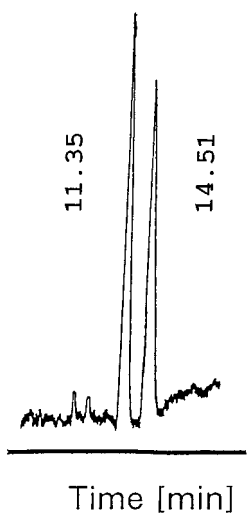
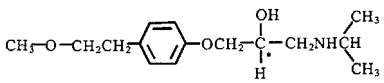


Figure 4. *Chiral separation of metoprolol. Conditions: column as in the legend to Figure 2; 10 mM α-PhoCD; buffer pH 8.3; voltage 15 kV.*



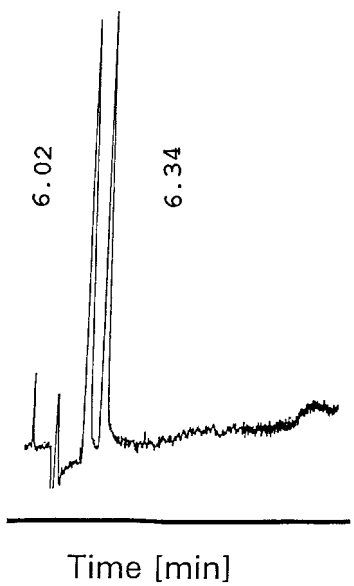


Figure 5. *Chiral separation of metoprolol. Conditions: column as in the legend to Figure 2; 15 mM α-PhoCD + 5% methanol; pH 8.3; voltage 20 kV.*

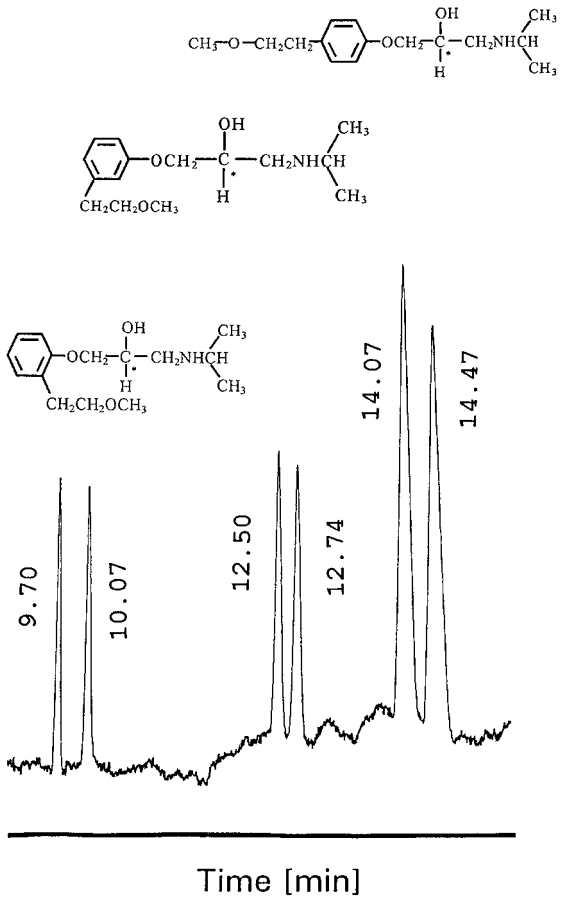


Figure 6. *Chiral separation of metoprolol and its ortho and meta by-products. Conditions: column as in the legend to Figure 2; 15 mM α-CMCD; pH 4.9; voltage 25 kV.*

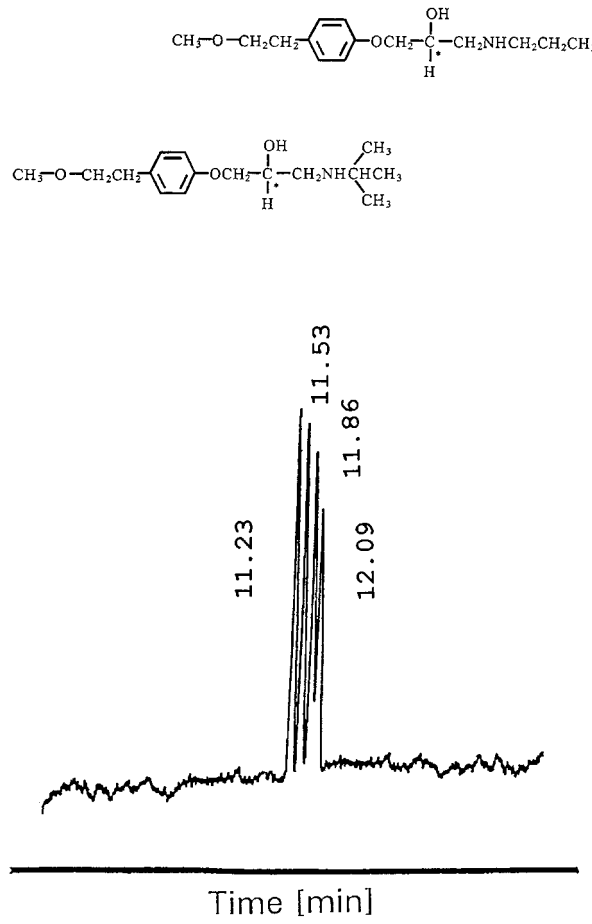


Figure 7. *Chiral separation of t-butyl and n-propyl by-products. Conditions: column as in the legend to Figure 2; 10 mM α-CMCD; pH 6.0; voltage 25 kV.*

prolol, *ortho* and *meta* by-products showed chiral resolutions similar to those observed with 15 mM α-CMCD at pH 4.9. The migration of metoprolol and *meta* isomers became closer. Using higher concentrations of α-CMCD, even better separations could be achieved for these enantiomeric pairs at pH 6. For example, 10 mM α-CMCD results in an R_s value of 4.3 for *ortho* and an R_s value of 1.8 for *meta*, but the metoprolol was not measurable, because it co-migrated with the system peak generated from the concentration decrease of CDs at the injection point. The use of 20 mM chiral separation agents produced an R_s value of 4.0 for *ortho* and an R_s value of 3.0 for metoprolol, but the enantiomeric separation of *meta* was not measurable, because in this case the *meta* co-migrated with the system peak. At pH 6, the *t*-butyl by-products were well separated from metoprolol (R_s 1.6), but their chiral resolution was smaller. On the other hand, the resolution of *n*-propyl improved. Even higher resolution values of 1.5 and 0.95 were achieved for *t*-butyl and *n*-propyl by-products, respectively, at pH 6 by decreasing the concentration of α-CMCD to 10 mM (Figure 7).

Below pH 4, the enantiomer selectivity was low toward metoprolol, and above pH 7, the *meta* by-product and main products were partly overlapping.

A concentration of 13 mM α-PhoCD produced an R_s value of 1.5 for the enantiomers of metoprolol, but the *ortho* isomers gave only an R_s value of 0.9 and the *meta* an R_s of 1.2 at pH 8.3. Under the same conditions, the peaks of *t*-butyl showed a good resolution (R_s value of 1.3), but its migration time was approximately the same as that for metoprolol. The *n*-propyl by-product was well separated from metoprolol, having a small chiral resolution (R_s 0.8). The urea by-product showed good chiral selectivity (R_s 1.2), but their peaks were mixed with the peaks of the meta by-product.

Using 10 mM γ-PhoCD, no chiral separation was observed in the cases of *ortho* and *meta* by-

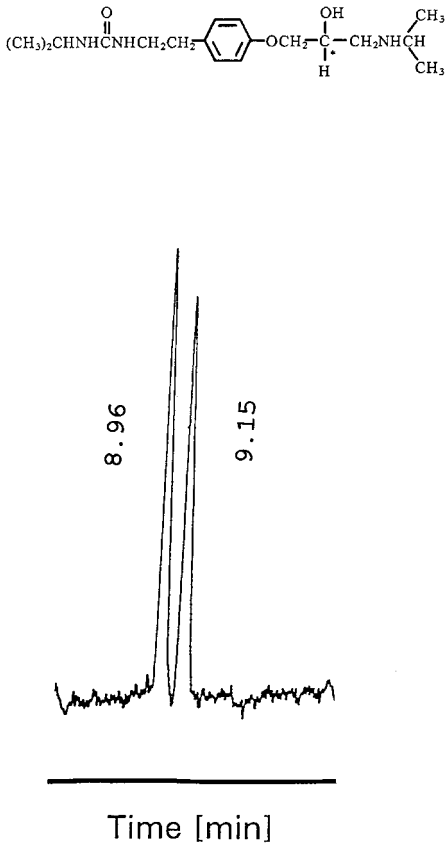


Figure 8. *Chiral separation of urea by-product. Conditions: column as in the legend to Figure 2; 5 mM γ-CMCD; pH 4.9; voltage 25 kV.*

products, but they separated well from metoprolol. The *t*-butyl by-product did not show chiral separation and, moreover, co-migrated with metoprolol. The *n*-propyl migrated well after the main compound, producing an R_s value of 0.8. Only the urea by-product showed improved chiral separation resulting in an R_s value of 1.5 (Figure 8) , migrating well after the other compounds.

The highest resolution values for the tested enantiomers are summarized in Table III with their corresponding analytical conditions.

Table III. *Investigated optimum conditions for the CE separation of metoprolol and its by-products*

Metoprolol	6	20	α-CMCD	20	3
ortho	6	10	α-CMCD	20	4.3
meta	6	10	α-CMCD	20	1.8
n-Propyl	6	10	α-CMCD	25	0.9
t-Butyl	6	10	α-CMCD	25	1.4
Urea	4.9	5	γ-PhoCD	25	1.6

CONCLUSION

Eight CD derivatives were found which showed chiral recognition toward metoprolol. Both selector and selectand have to be charged to give an R_s value of 1.5. α-CMCD followed by α-PhoCD are the best chiral selectors for appropriate separation of by-products from metoprolol as well as for their chiral recognition.

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