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Capillary zone electrophoretic separation of (*R*,*S*)-metipranolol and related substances

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Capillary zone electrophoresis was used for separation and quantitation of impurities occuring in the raw metipranolol. Sufficient separation of peaks of impurities in concentrations below 1% from that of the active ingredient was only possible in the presence of cyclodextrins, the highest resolution was observed in the presence of γ-CD and HP-β-CD without chiral discrimination of the enantiomers. Pyridoxol was used as an internal standard in quantification. Enantiomers of metipranolol and desacetylmetipranolol were resolved in the presence of carboxymethylated β-CD; the highest resolution was observed in the presence of 20 mM of CY-β-CD, 25 kV and 18 °C.

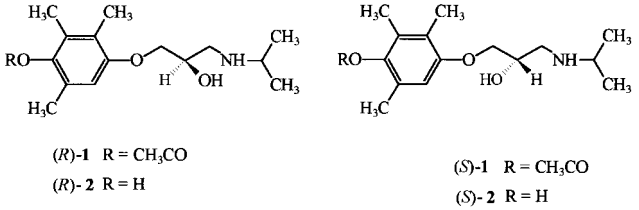
1. Introduction

(*R*,*S*)-Metipranolol (**1**), an aryloxipropanolamine non-selective β-blocker is used in the therapy of cardiovascular disorders and in ophthalmology. So far, compounds of this therapeutic group were analyzed predominantly by HPLC and CE. Methods with or without derivatization were developed for the separation of enantiomers of β-blockers by HPLC: the former used esterification of (*R*,*S*)-propanolamines with enantiomeric pure tartaric acid, the resulting diastereomeric monoesters were separated by RP-HPLC [1]. Adsorption LC was used for the separation of diastereoisomers prepared by reaction of β-blockers with (−)-camphanic acid chloride, or chiral isocyanates [2]. The latter used chiral LC columns packed with e.g. modified cellulose (Chiralcel OD) [3, 4], polyacrylamide (ChiraSpher), derivatives of cyclodextrin (Cyclobond), silica with bonded proteins [4, 5], or Pirkle type chiral stationary phases [6]. CE separation of enantiomers of β-blockers used modified cyclodextrins alone [7–11] or other comodifiers such as pyrene forming a ternary complex with analyte and cyclodextrin [12]. Another approach used the completion of an electrolyte with chiral polymers: cross-linked protein-gel (cellobiohydrolase) [13], carrageenan [14] or human serum transferrin [15]. Micellar electrokinetic chromatography (MEKC) in the presence of (*R*)- or (*S*)-*N*-dodecoxycarbonylvaline was used for the chiral discrimination of a series of propanolamines and other basic drugs [16].

In the present work, the separation of metipranolol from its related substances, degradation- and by-products of its synthesis as well as the separation of enantiomers of metipranolol and desacetylmetipranolol was studied by CZE in the presence of cyclodextrins.

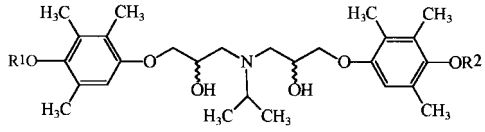
2. Investigations, results and discussion

The main degradation product and the first metabolite of (*R*,*S*)-metipranolol (**1**) is desacetylametipranolol ((*R*,*S*)-**2**), compounds **3** and **6** represent by-products of the synthesis of the active drug. Compounds **1**–**3** and **6** were sufficiently separated by capillary zone electrophoresis (CZE) in a plain acidic buffer in the longer capillary (56 cm, Fig. 1) as well as in the shorter one (40 cm) which was used in the further experiments. The resolution of peaks of **1** and **2** varied with the type of the background electrolyte (BGE, Fig. 2) and reached the maximum ($R_s = 2.7$) at pH 2.8 in 100 mM TRIS/phosphate buffer. However, this resolution was not sufficient for a determination of **2** in

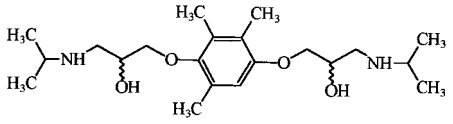


(*R*)-**1** R = CH_3CO
(*R*)-**2** R = H

(*S*)-**1** R = CH_3CO
(*S*)-**2** R = H



3 R1 = R2 = CH_3CO
4 R1 = H R2 = CH_3CO
5 R1 = R2 = H



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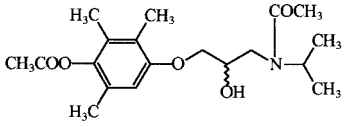

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samples of raw metipranolol (**1**) where the mass ratio of **1** : **2** was higher than 50. The addition of cyclodextrins except CY-β-CD and CY-γ-CD into BGE at pH 2.8 enhanced the resolution of peaks of these compounds in comparison with the plain BGE (Fig. 3); these selectors lowered resolution, however, only in their presence a splitting of peaks of compounds **1** and **2** was observed, indicating the chiral discrimination of (*R*)- and (*S*)-enantiomers. The resolution of peaks of **1** and **2** was highest in the presence of 5 mM of γ-CD and HP-β-CD ($R_s = 4.25$ and 3.90, respectively) which was sufficient for a reliable separation of peaks of **1** and **2** in a concentration range of 0.1–1.0% desacetylmetipranolol (**2**) in raw metipranolol (**1**) (Fig. 4).

The addition of an internal standard (pyridoxol) proved to be advantageous for a proper quantification of the studied compounds in raw metipranolol at concentrations below

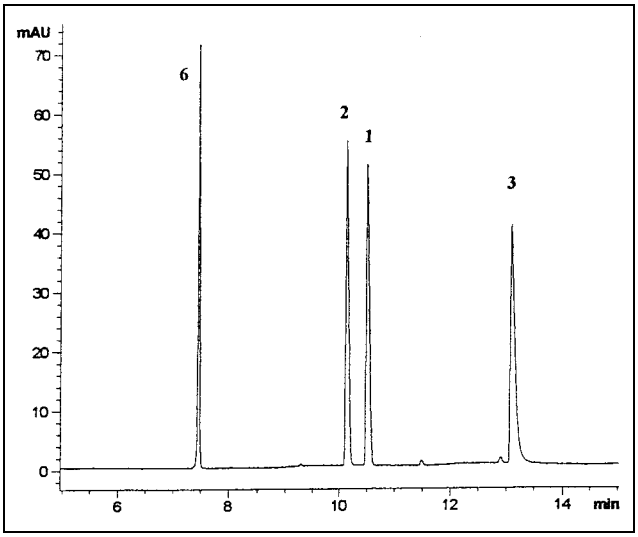

Fig. 1: Separation of metipranolol (**1**) and compounds **2**, **3** and **6**. Capillary 56 cm × 0.05 mm, 100 mM TRIS/phosphate buffer pH 2.70, 30 kV, 25 °C

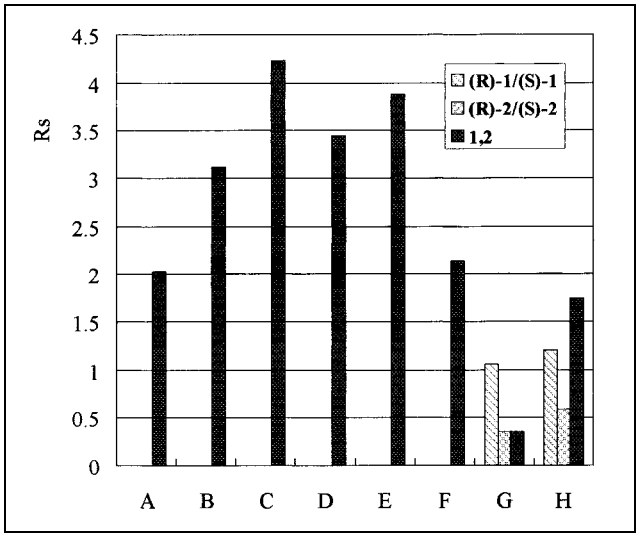

Fig. 3: Resolution of peaks of metipranolol (**1**) and desacetylmetipranolol (**2**) in the presence of cyclodextrins (5 mM). Capillary 40 cm × 0.05 mm, 100 mM TRIS/phosphate, pH 2.80, 30 kV, 25 °C. A: without CD; B: D-β-CD; C: γ-CD; D: HP-γ-CD; E: HP-β-CD; F: β-CD; G: CY-γ-CDX; H: CY-β-CD

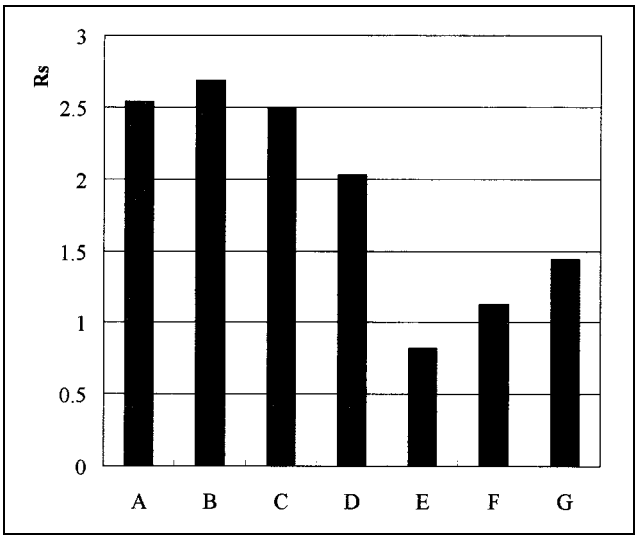

Fig. 2: Resolution (R_s) of metipranolol and desacetylmetipranolol in various background electrolytes. Capillary: 40 cm × 0.05 mm, 30 kV, 25 °C. A: 80 mM TRIS/phosphate, pH 2.80; B: 100 mM TRIS/phosphate, pH 2.80; C: 80 mM TRIS/phosphate, pH 3.38; D: 50 mM tartaric acid/TRIS, pH 4.50; E: 50 mM AcOH/AcONa, pH 5.08; F: 50 mM MES/TRIS, pH 6.50; G: 50 mM tartaric acid/TRIS, pH 6.50

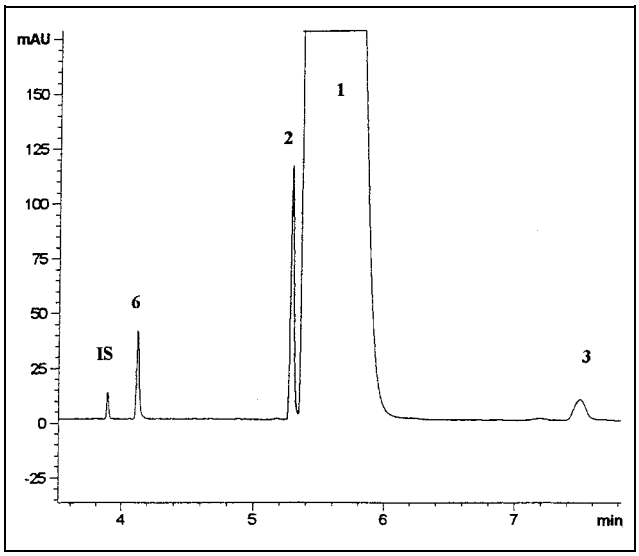

Fig. 4: Electrophoretogram of metipranolol (**1**) with spiked impurities **2** (0.42), **3** (0.15); IS-pyridoxol (0.07 mg/ml). Capillary 40 cm × 0.05 mm, 100 mM TRIS/phosphate buffer pH 2.70, 5 mM HP-β-CD, 30 kV, 25 °C

1% because a higher precision was achieved. The detection limit of the separated compounds was 0.4–1.6 μg/ml; the calibration curve, determined by analysis at 10 concentration levels was linear in the range 0.005–0.5 mg/ml for compounds **2**, **3** and **6** with a regression coefficient better than 0.99.

The precision expressed by the repeatability was determined by analysis of 3 samples (10 injections) of metipranolol solutions spiked with impurities **2**, **3** and **6** and pyridoxine as an internal standard with results (% RSD) as follows: migration time 1.89, 1.77, 1.82, 1.90 and 1.72, resolution of peaks 1.81, 1.62, 1.76, 1.3%, ratio of peak area vs. area of IS 1.06, 3.81, 1.08 and 1.94% for the *O*,*O*-bisderivative **6**, **2**, **1** and the *N*,*N*-bisderivative **3**, respectively.

Similarly to **1** the *N*,*N*-bisderivative **3** is hydrolyzed into the phenols **4** and **5**. The alkaline hydrolysis of **3** was monitered by CZE; all peaks were well resolved also in the plain BGE (Fig. 5). Both hydrolyzates were identified in samples of raw metipranolol only in concentrations below 0.1%.

Metipranolol (**1**) refluxed in 2-propanole afforded the N-amide **7** and desacetyltrimepranol (**2**) by intermolecular transacetylation. Because compound **7** was not ionized in the BGE used, it was transported by electroosmotic flow and its peak appeared at 21 min; however, this reaction was reliably monitored by CZE (Fig. 6).

The separation of enantiomers of (*R,S*)-metipranolol (**1**) in the presence of cyclodextrins depends on pH and type of BGE. A comparison of various CDs at a concentration of 5 mM in 80 mM triethanolamine/phosphate buffer in a 40 cm capillary revealed that only carboxymethylated CDs discriminated the enantiomers; however, with the enantiomers of **2** a higher chiral selectivity was observed than with those of the parent compound **1**. This resolution was dependend on the concentration of CD; the apex of this

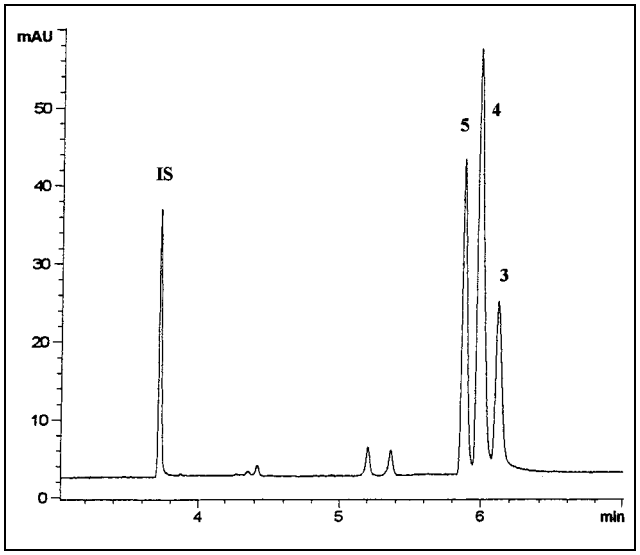

Fig. 5: Separation of the *N,N*-bisderivative **1** and its deacetylated products **4** and **5**, IS-pyridoxol. Capillary 40 cm × 0.05 mm, 100 mM TRIS/phosphate buffer pH 2.70, 30 kV, 25 °C

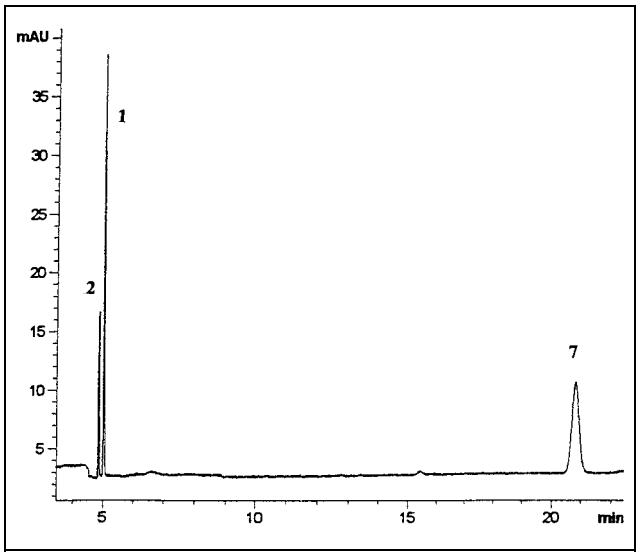

Fig. 6: Separation of metipranolol (**1**) desacetylmetipranolol (**2**) and N-amide **7**. Capillary 40 cm × 0.05 mm, 100 mM TRIS/phosphate buffer pH 2.70, 30 kV, 25 °C

function was at 20 mM of CY-β-CD. It is remarkable that both enantiomers of **2** formed more stable complexes with CY-β-CD than **1** did (higher migration times of enantiomers of **2** than those of **1**, however, the stability of the complexes reversed with an apparent lower resolution of peaks observed in the presence CY-γ-CD; Figs. 7, 8). The former CD separated also the diastereoisomers of the *O,O*-bisderivative **6** (Fig. 7).

Because the resolution of peaks of (*R*)- and (*S*)-**2** was higher than that of (*R*)- and (*S*)-**1** (R_s of their peaks did not exceed 1.4), samples of **1** were hydrolyzed by methanolic KOH under nitrogen into the phenol **2** prior to the determination of the optical purity of **1** prepared by crystallization of diastereoisomeric salts with *R*- or *S*-mandelic acid, or synthesized from *R*- or *S*-epichlorohydrine. The alkaline hydrolysis of **1** was completed in approx. 30 min at room temperature, or in 10 min at 40 °C.

The resolution of peaks of (*R,S*)-**2** or (*R,S*)-**1** was dependent of the voltage applied and the temperature of the capillary. A maximum of this function was seen at about 25 and 20 kV for (*R,S*)-**2** amd (*R,S*)-**1**, respectively (Fig. 9).

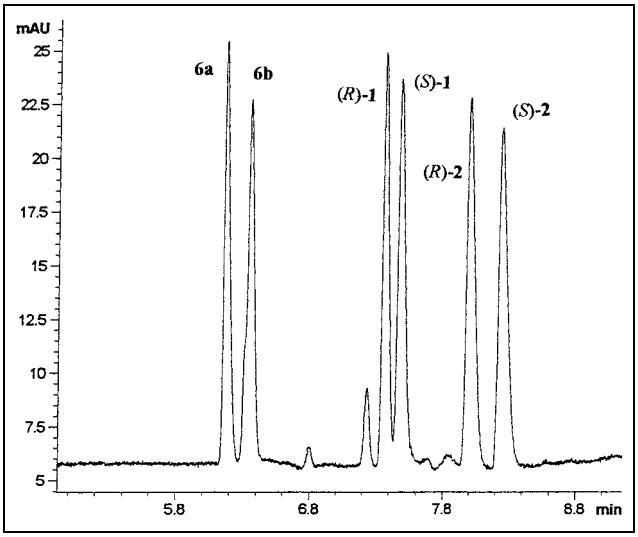

Fig. 7: Separation of diastereoisomers of the *O,O*-bisderivative **6a**, **6b**, (*R*)/(*S*)-metipranolol ((*R*)-**1**, (*S*)-**1**) and (*R*)/(*S*)-desacetylmetipranolol ((*R*)-**2**, (*S*)-**2**). Capillary 40 cm × 0.05 mm, 80 mM triethanolamine/acetate buffer pH 5.70, 20 mM CY-β-CD, 25 kV, 18 °C

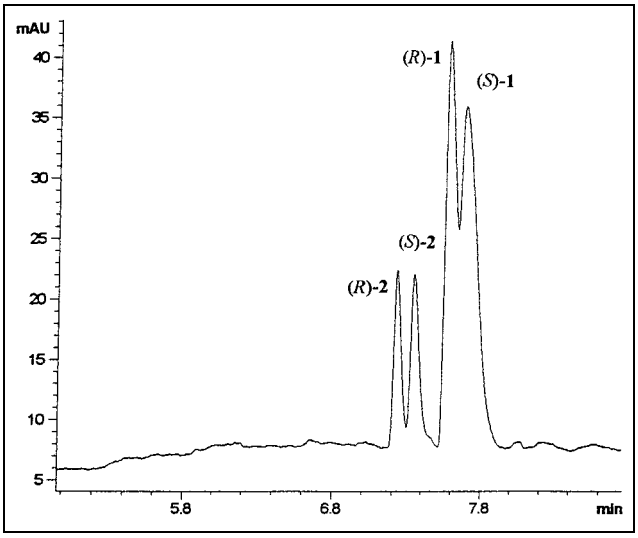

Fig. 8: Separation of (*R*)/(*S*)-desacetylmetipranolol ((*R*)-**2**, (*S*)-**2**) and (*R*)/(*S*)-metipranolol ((*R*)-**1**, (*S*)-**1**). Capillary 40 cm × 0.05 mm, 80 mM triethanolamine/acetate buffer pH 5.70, 20 mM CY-β-CD, 25 kV, 18 °C

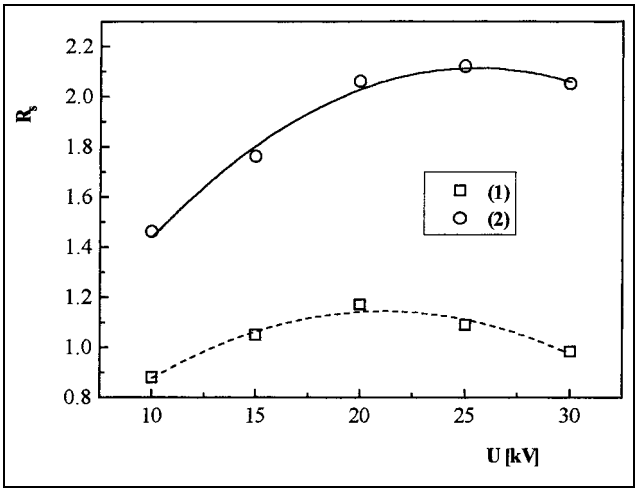

Fig. 9: Resolution (R_s) of peaks of (*R*)/(*S*)-metipranolol (**1**) and (*R*)/(*S*)-desacetylmetipranolol (**2**) as a function of the voltage applied. Capillary 40 cm × 0.05 mm, 80 mM TEA/acetate pH 5.70, 20 mM CY-β-CD, 25 °C

3. Experimental

A HP ^{3D}Capillary Electrophoresis apparatus (Hewlett Packard, Waldbronn, Germany) with a diode array detector (190–600 nm) was used for analysis. The electrophoretograms were collected at a fixed UV wavelength of 215 nm with data processed on a HP ChemStation. Capillaries: untreated fused silica capillary tubes 48.5 and 64.5 cm (effective length 40 and 56 cm, respectively) with 0.05 mm ID and an extended light path (3×) were used. Prior to use, the bare silica capillary was rinsed with 1 M NaOH (20 min), distilled H_2O (15 min) and the appropriate BGE (10 min). Between analyses, the capillaries were flushed with 10 mM H_3PO_4 (1 min), distilled H_2O (1 min) and a buffer solution (2 min). Samples were kept at laboratory temperature in the autosampler and injected under pressure at 5 kPa for 2 s. The resolution of peaks was calculated according to equation $R_s = 2(t_2 - t_1)/(w_1 + w_2)$ where t = migration times, w = baseline peak width.

(*R*)- and (*S*)-metipranolol, (*R*)- and (*S*)-**1**, respectively, (*R*)- and (*S*)-desacetylmetipranolol, (*R*)- and (*S*)-**2**, respectively; *N,N*-bisderivative **3**; *O,O*-bisderivative **6** and *N*-acetylmetipranolol **7** were prepared in Slovakofarma a.s. and their structures were confirmed by spectral methods.

β-Cyclodextrin (β-CD), γ-cyclodextrin (γ-CD), 2-hydroxypropyl-β-cyclodextrin (HP-β-CD), 2-hydroxypropyl-γ-cyclodextrin (HP-γ-CD), heptakis (2,6-di-O-methyl)-β-cyclodextrin (D-β-CD), carboxymethyl-β-cyclodextrin (CY-β-CD), carboxymethyl-γ-cyclodextrin (CY-γ-CD) were supplied by Cyclolab, Budapest, Hungary. All other chemicals were purchased from Fluka AG, Buchs, Switzerland.

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