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Determination of the enantiomeric purity of (−) terbutaline by capillary electrophoresis using cyclodextrins as chiral selectors in a polyethylene glycol gel

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Abstract

A method was developed for determination of the enantiomeric purity of the therapeutic-pharmacological active (−)-enantiomer of terbutaline using cyclodextrins as a chiral selector dissolved in a removable liquid polyethylene glycol gel by use of capillary electrophoresis. The effect of temperature, type and concentration of polyethylene glycol and cyclodextrins was studied on the resolution between the two enantiomers. Best results were obtained with 10 mM hydroxyethyl-β-cyclodextrin dissolved in a 10% polyethylene glycol-2000 solution at 15°C. Under these conditions, an impurity of 0.1% (distomer/eutomer) can be readily detected.

Keywords: Enantiomeric purity; Cyclodextrins; Polyethylene glycol; Capillary electrophoresis; Terbutaline hemisulphate

1. Introduction

Enantiomers of racemic drugs often differ in their pharmacokinetic behaviour and/or pharmacological action [1] (Fig. 1).

Terbutaline, a sympaticomimetic drug-selective β_2-receptor agonist is used in the treatment of asthma and lung diseases. When administered in large doses it can be used as a growth promoter to improve meat-to-fat ratios in cattle. At high concentrations in liver or in meat, residues of these compounds are toxic to humans, leading to sickness and possible heart complications. Therefore they are not authorized for use as a growth promoter in the European Union.

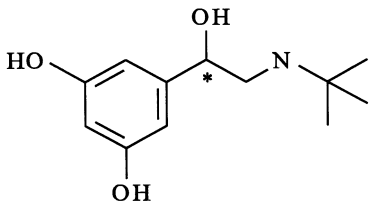

Fig. 1. The chemical structure of terbutaline, * indicates the chiral centre.

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The drug is usually administered as a racemate, but studies have shown that only the (−) enantiomer has the desired therapeutic pharmacological effect [2]. For that reason it is of great importance that the enantiomers of such molecules can be separated. Basic enantiomers like terbutaline can be separated by capillary electrophoresis using cyclodextrins as a chiral selector [3–9]. Also other chiral selectors like macrocylic antibiotics [10], cellulase [11], sodiumdodecylsulphate micelles [6], crown ethers [6,12] or combinations of the former [13] can be used for this purpose. Cyclodextrins are commercially available oligosaccharides of 6,7 or 8 D-(+)-glucopyranose units and they are designated as α-, β- and γ-CD. Derivatization of the plain cyclodextrins leads to numerous types of cyclodextrins, all with their own stereospecificity. Especially in the case of β-CD, derivatization leads to a better solubility [14]. Chiral recognition with cyclodextrin (CD) is based on a few acquirements as suggested by Armstrong et al. [15]: (1) an inclusion complex must be formed, (2) a tight fit of the included species with the host cavity must occur (3) the stereogenic centre of the analyte should be able to form one strong interaction with the hydroxyl groups of the CD cavity entrance and (4) the unidirectional 2- or 3-hydroxyl groups at the second face of the macrocycle are especially important for chiral recognition.

The stability of the formed complexes depends on the degree of fit of the more or less apolar side-chain of the host-molecule and the hydrophobic cavity of the CD.

Cyclodextrins dissolved in liquid or immobilized polyacrylamide (PAG) gels, solid bovine serum albumine (BSA) gels, liquid crown ether gels or polyethylene glycol gels seem to improve the chiral separation as a result of a decrease in the adsorption of the analyte(s) to the capillary wall [14,16–21]. A big advantage of the use of liquid gels, which were added to the ground electrolyte, is that the capillaries used, generally have a longer lifetime [22].

This paper describes a method for the determination of the enantiomeric purity of (−)-terbutaline by systematical research of the effect of the CD type and its concentration, the PEG type and its concentration and the influence of the temperature on the enantiomeric separation of racemic-terbutaline. The capillary was filled with cyclodextrins dissolved in a removable liquid polyethylene glycol (PEG) gel, in order to improve the selectivity of the chiral separation and to extend the useful lifetime of the capillary, but also to decrease the total amount of chiral selector necessary for one separation.

2. Experimental

2.1. Apparatus

The CE system was a Model PRINCE with a 4 position sample tray and a programmable injector system from Lauerlabs (Emmen, The Netherlands). Detection at 210 nm was carried out with a LAMBDA 1000 UV/VIS VWL detector (Bischoff, Leonberg, Germany). The bare fused-silica capillary with an outer polyimide coating (50 μm i.d., 375 μm, o.d.) was from Polymicro (Phoenix, AZ). Data acquisition of CE/UV was performed by the Maclab system (ADinstruments, Castle Hill, Australia) using the Chart program (version 3.3, ADinstruments) for recording the electropherograms. For interpretation of the electropherograms, the Peaks program (ADinstruments) was used. The vials used were 4 ml glass vials sometimes with a 0.7 ml plastic insert for a Waters 96 and were obtained from Phase Sep (Waddinxveen, The Netherlands).

2.2. Solutions

The CE run-buffer was prepared by dissolving sodium dihydrogen phosphate monohydrate (Merck, Darmstadt, Germany) to a concentration of 100 mM and adjusting the pH with concentrated *ortho*-phosphoric acid (85%, Merck) to a pH of 2.5 and a conductivity of 7.0 mS cm^{-1}. β-Cyclodextrin (β-CD) was obtained from Serva (Heidelberg, Germany), heptakis (2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD) and hydroxyethyl-β-cyclodextrin (HE-β-CD) were obtained from Wacker, Germany.

Polyethylene glycol 300 (PEG-300) was obtained from Brocacef (Maarssen, The Netherlands), PEG-2000 was from Merck and PEG-6000 was from Genfarma (Maarssen, The Netherlands). Water was purified with a Milli-Q system (Millipore, Bedford, MA). The conductivity of the purified water was always less than 2 μS cm^{-1}.

Terbutaline hemisulphate racemate (Pharmacopoeial quality) was dissolved in a solution containing one part run-buffer and nine parts of water to a final concentration of 20 μg ml^{-1}.

(−) Terbutaline and (+) terbutaline were separated by HPLC according to the procedure of Walhagen et al. [23] using 5/95 (v/v) methanol (Merck)/0.025 M citric acid buffer as the mobile phase. The citric acid buffer was prepared by dissolving citric acid monohydrate (Merck) to a concentration of 0.025 M adjusting the pH to 6.0 with a 1 M sodiumhydroxide (Merck) solution. Separation took place at 4°C. Under these conditions a full baseline separation was achieved, resulting in a resolution of 1.2. (−) Terbutaline was collected and concentrated using a Solid Phase Extraction according to the previous published method of Vyncht et al. [24]. The SCX cation-exchange columns (Analytichem, Harbor City, CA) were preconditioned with 5 ml methanol, 5 ml water and 5 ml 0.01 M potassiumdihydrogen phosphate (pH = 3.0). After addition of the sample that was adjusted to pH 3.0 with concentrated phosphoric acid, the column was washed with 2 ml 0.01 M potassiumdihydrogen phosphate (pH = 3.0) and 2 ml methanol. After drying under depression, (−)-terbutaline was eluted under gravity with 2 ml of a methanol/ammonia solution (95:5, v/v).

The extract was dried under a nitrogen stream at room-temperature and dissolved in 300 μl of a solution containing one part run-buffer and nine parts of water and then spiked with racemic terbutaline for impurity experiments.

All solutions were filtered through a membrane filter (0.45 μm) and degassed for 5 min in an ultra-sonic bath (50 kHz, Branson Europa, Soest, The Netherlands), immediately prior to use.

2.3. CE conditions used for experiments

A capillary with a total length of 70 cm and an effective length of 55 cm was used. An optical viewing window with a length of 0.5 cm, obtained by burning off the polyimide coating, was aligned with the UV detection cell. The coating of the first 2 mm of the capillary was also stripped.

New capillaries were rinsed with 1 M sodium hydroxide for 10 min at 1000 mBar, with water for 10 min at 1000 mBar and with the runbuffer for 10 min at 1000 mBar.

When cyclodextrins and Polyethylene glycol were used for separation, they were dissolved in the runbuffer and hydrodynamically injected as a removable gel until the capillary was fully filled. The latter was monitored by UV-detection. The analyte then was injected electrokinetically in order to avoid the PEG/CD gel to be forced out. The injection (10 kV, 6 s) and separation voltage (30 kV) were ramped at 6 kV s^{-1}. After each run the capillary was refilled with de PEG/CD gel. Because we inject the removable liquid gel directly into the capillary instead of adding it to the ground-electrolyte, the electro-osmotic flow (EOF) should be suppressed to avoid the removable gel to be forced out of the capillary. The latter is partially accomplished by the increased viscosity, but a working pH of the ground-electrolyte of pH < 3 will generally eliminate the EOF. For the same reason the analyte(s) can only be introduced into the capillary by electrokinetic injection, which implies that although electrokinetic injection increases sensitivity due to stacking, some precautions with respect to the amount of injected analyte(s) should be taken into account [25–29].

Separations were carried out after the electrode and the capillary-end were dipped in a vial containing water and began when the ground electrode and the capillary-end were placed into the vial containing the run buffer.

2.4. Statistical methods used for experiments

One-way analysis of variance (ANOVA), the paired and independent two-sample *t*-test and

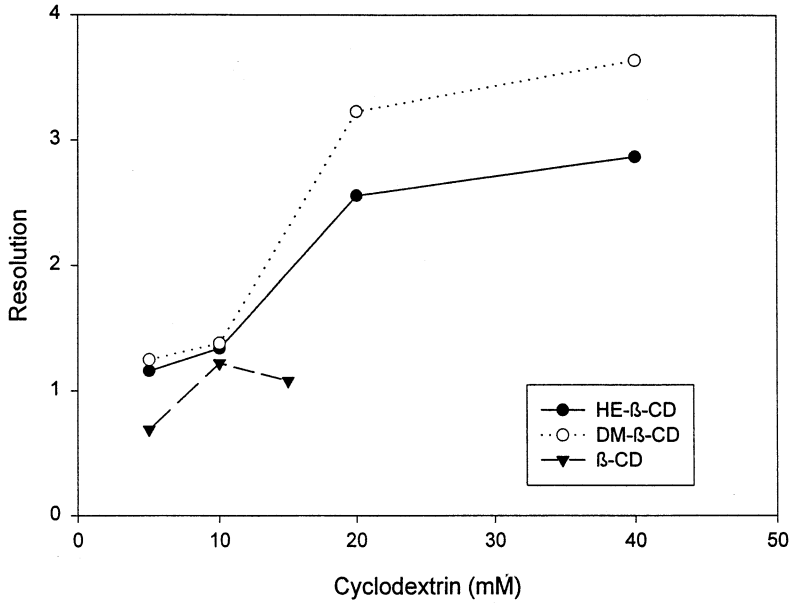

Fig. 2. Effect of cyclodextrin concentration on resolution of racemic-terbutaline.

linear regression were performed with an Origin 3.0 (MicroCal, Northampton, MA) program.

3. Results and discussion

3.1. Type and concentration of cyclodextrin

In Fig. 2, the resolutions for (+)- and (−)-terbutaline are presented as a function of the molarity of three different types of cyclodextrins. Because the solubility of β-CD in aqueous solutions is low, concentrations of β-CD > 15 mM cannot be prepared and therefore not be compared with DM-β-CD and HE-β-CD in this matter. According to the data obtained, the resolution shows an increase when the CD-concentration increases, this is due to an increase in complexation with the analytes. Furthermore, the derivatized β-cyclodextrins are more chiral selective, probably as a consequence of the less apolar side-chain of the host molecule in combination with the molecular structure of the analyte.

The highest resolutions were obtained with DM-β-CD and ranged from 1.25 to 3.64 (~191%) for concentrations of DM-β-CD ranging from 5 to 40 mM.

3.2. Type and concentration of polyethylene glycol

To explore the influence of the removable gel, hydroxyethyl-β-cyclodextrin was used in a concentration of 10 mM for further experiments. At this concentration the resolutions obtained with HE-β-CD and DM-β-CD are nearly the same and for practical reasons further experiments were carried out with HE-β-CD. It was therefore dissolved in a removable polyethylene glycol (PEG) gel and injected hydrodynamically into the capillary until it was fully filled. In Fig. 3 the resolutions are presented as a function of the percentage PEG with different chain lengths. For PEG-300 resolutions range from 1.17 (0% PEG) to 1.57 (5% PEG), which corresponds to a maximum increase of ~34%. An independent two-sample t-test of the data confirms that there is a significant difference between the means at a 95% reliability level ($\alpha = 0.05$; $t = 2.6101$, $p = 0.04012$): where α = the error of the first kind (unreliability in accepting the null-hypothesis); F = probability distribution (the ratio of two independent variance estimates obtained from the sample normal distribution); and p = probability factor (the factor that indicates the chance that the given test-statistic is not correct).

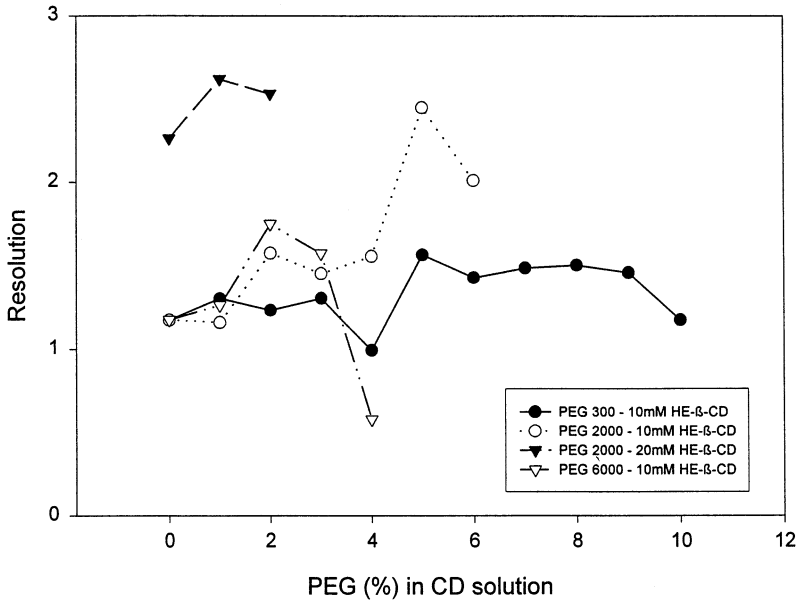

Fig. 3. Effect of the polyethyleneglycol (PEG) concentration in different types of cyclodextrin on the resolution of racemic-terbutaline.

Similar experiments were made with PEG-6000; resolutions are plotted in Fig. 3 as a function of the concentration of the polyethylene glycol. For PEG-6000 resolutions range from 1.17 (0% PEG) to 1.75 (2% PEG), which corresponds to a maximum increase of ~49%. An independent two-sample t-test of the data confirms that there is a significant difference between the means at a 95% reliability level. ($\alpha = 0.05$; $t = 3.3640$; $p = 0.01515$).

When 10 mM HE-β-CD is dissolved in a solution containing PEG-2000 resolutions range from 1.17 (0% PEG) to 2.45 (5% PEG), which corresponds to a maximum increase of ~109%. An independent two-sample t-test of the data confirms that there is a significant difference between the means at a 95% reliability level. ($\alpha = 0.05$; $t = 3.98936$; $p = 0.01043$).

The resolutions obtained with a 20 mM HE-β-CD dissolved in a PEG-2000 solution are also plotted in Fig. 3. The maximum increase in resolution here is 16%; an independent two-sample t-test does confirm that there is a significant difference between the means at a 95% reliability level. ($\alpha = 0.05$; $t = 3.96968$; $p = 0.00061$).

It seems that addition of polyethylene glycol to the cyclodextrin solutions has a positive effect on the resolution using low concentrations of cyclodextrins, but beyond a certain concentration of polyethylene glycol, that depends on the type of polyethylene glycol used, the baseline tends to fluctuate and makes it impossible to determine the resolution. This phenomenon might be dependent on fluctuations in temperature. An other phenomenon is an optimum in resolution also depending on the type of polyethylene glycol used. Both phenomena might be due to a change in conformation of the polyethylene glycol, but cannot be explained properly at the moment.

3.3. Effect of temperature on chiral separation

The next step in optimizing was to monitor the influence of the temperature on chiral separation. For this reason 10 mM HE-β-CD was incorporated in a PEG-2000 gel. Table 1. shows that for chiral separation using cyclodextrins, the resolution increases when the temperature decreases.

Table 1
Effect of temperature on resolution of racemic terbutaline using different concentrations PEG-2000 dissolved in a 10 mM HE-β-CD solution

PEG-2000 (%)	15°C		20°C		25°C	
	Rs	R.S.D. (%)	Rs	R.S.D. (%)	Rs	R.S.D. (%)
0	1.73[a]	7.5	0.94[a]	18	0.82[a]	35
1	1.87	5.3	1.21	9.1	1.00	43
2	1.92	6.0	1.38	11	1.20[b]	2.4
3	2.00	6.1	1.24	34	0.97	32
4	1.93	8.9	1.20	5.2	1.04	11
5	1.97	2.9	1.25	14	1.04	10
7	2.05	12	1.50[b]	20	0.95	17
10	2.42[b]	13	1.38	24	1.09	15
15	2.33	11	1.43	4.7	1.06	3.5
Max increase	~40%		~60%		~46%	

R.S.D. corresponds to the relative standard deviation and is given by the quotient of the standard deviation and the mean.
[a] Depicts the lowest obtained resolution.
[b] The highest obtained resolution.

The latter can be explained by the fact that decreasing the temperature will result in an increase of the viscosity and therefore a decrease of the mobility of the enantiomers resulting in an increase in the difference between the complex formation constants of the two enantiomers. The improved temperature control considerably reduced the baseline fluctuations observed in the preliminary experiments. Therefore we were able to test concentrations of PEG-2000 up to 15%

Table 1. shows the resolutions obtained with different concentrations of polyethylene glycol at different temperatures. In the table the maximum and minimum resolutions along with the maximum increase are indicated. The highest resolution occurs when 10% polyethylene glycol is dissolved in 10 mM HE-β-CD at 15°C.

Fig. 4. Visualizes the increase of the resolutions when decreasing the temperature. It shows that the reduction in temperature can give rise to a more than 100% increase in resolution. The effect of temperature on resolution is also visualized in the electropherograms in Fig. 5.

3.4. Enantiomeric impurity profiling of (−)-terbutaline

A typical detection limit for enantiomeric impurities monitored by CE is 0.1% (area of distomer/area of eutomer) [30–33]. The enantiomeric impurity of (−)-terbutaline was calculated by internal normalization from corrected peak areas (peak area divided by the corresponding migration time) as suggested by Altria [30]. The resolu-

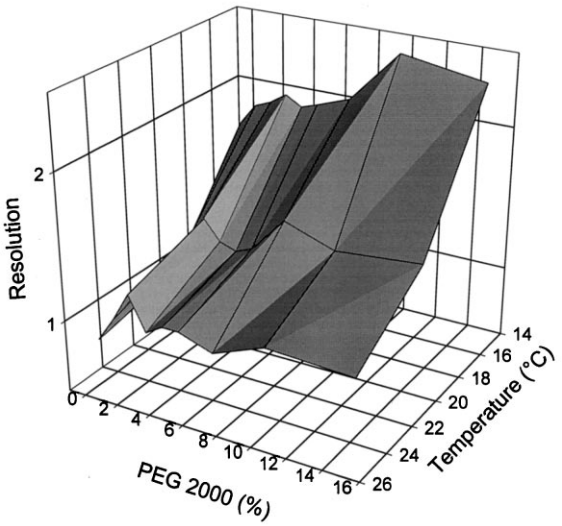

Fig. 4. Effect of the percentage PEG-2000 in 10 mM HE-β-CD solution of racemic-terbutaline at different temperatures.

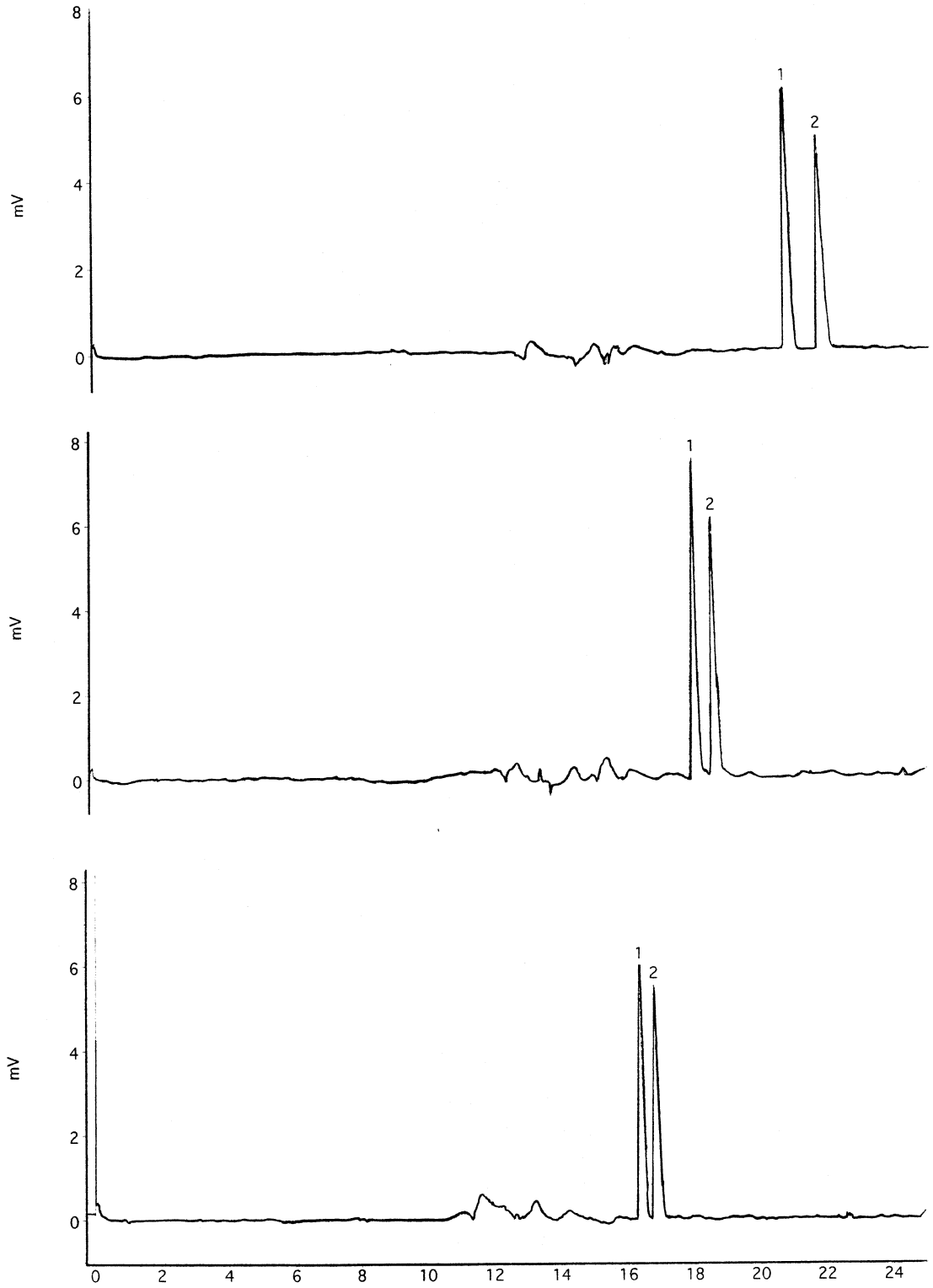

Fig. 5. Electropherograms of separation of racemic-tebutaline at different temperatures (15, 20 and 25°C, respectively) using 10% PEG-2000 in 10 mM HE-β-CD as a removable gel.

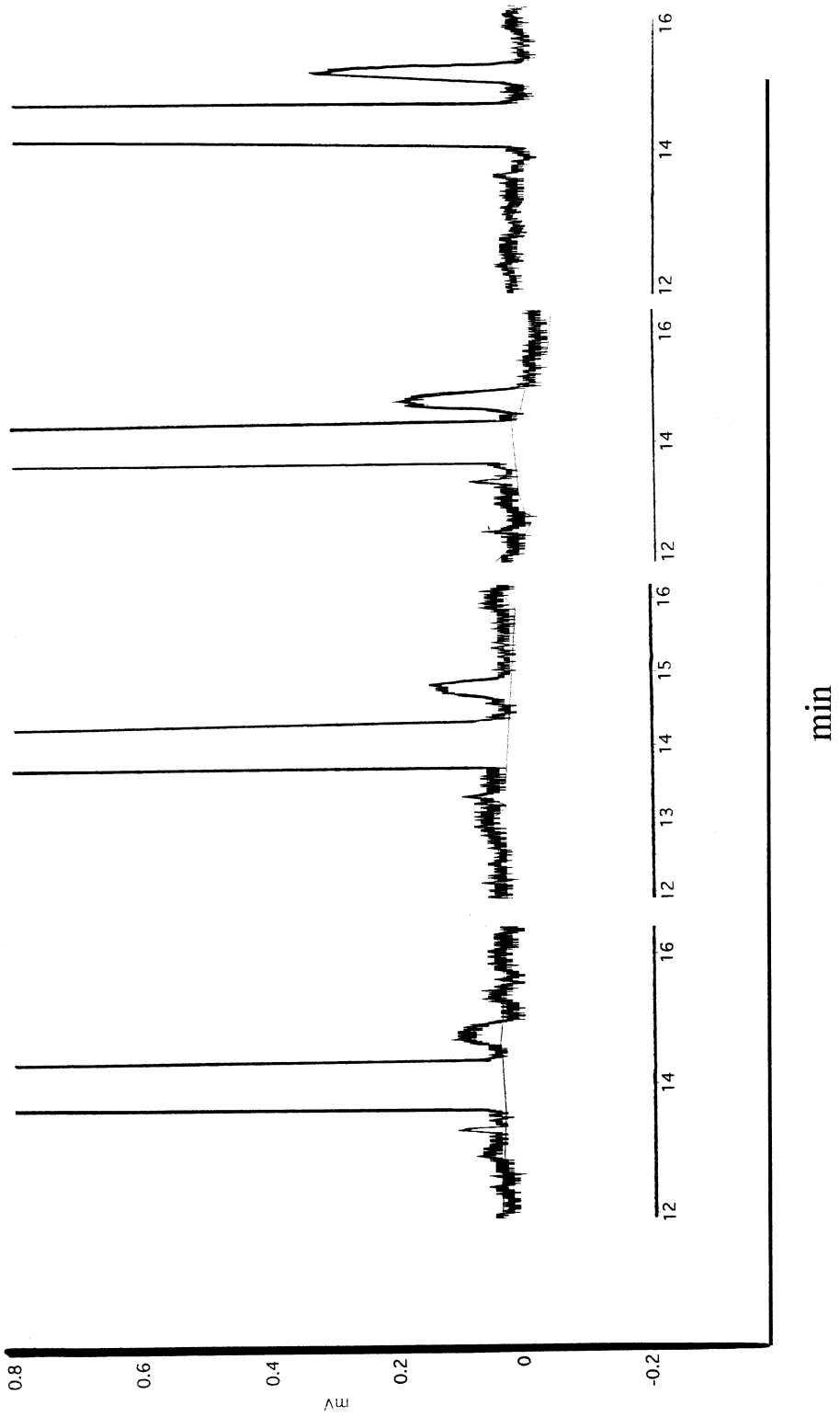

Fig. 6. Electropherograms of 0.1, 0.5, 0.7 and 1.3% spiked (+)-terbutaline using 2% PEG-2000 in 10 mM HE-β-CD as a removable gel. Electrokinetic injection, 30 kV, 12 s; separation, 30 kV.

tion, that is defined as the extent of separation between two compounds is generally considered to be adequate when $R = 1.5$ (less than 0.2% overlap for symmetrical comparable peaks). To accomplish a full baseline separation to allow impurity studies up to 0.1% a resolution of 2.5 is necessary, as can be calculated from the theoretical overlap of symmetrical peaks by simple overlap studies.

When a resolution of 2.5 is achieved, the contribution of the main component ((−)-terbutaline) to the peak area of the impurity ((+)-terbutaline) is less than 0.2% for symmetrical peaks. The purity of the collected (−)-terbutaline was tested by the developed CE method. No trace of the (+)-enantiomer was observed (< 0.1%).

Fig. 6. shows some representative electropherograms of (−)-terbutaline spiked with low levels of (+)-terbutaline. The limit of detection (L.O.D.) observed was 0.1% impurity of (+)-terbutaline.

In order to illustrate the linearity of the detector response, linear regression analysis ($Y = a + bX$) was performed and gave in the range of 0.1–1.3% (+)-terbutaline, the following equation: $Y = 0.021(\pm 0.006) + 0.0243X \quad (\pm 0.0007)$ corresponding with an adequate linearity, expressed as the coefficient of determination ($r^2 = 0.998$; $p = 0.0009$; $n = 4$).

4. Conclusion

The use of cyclodextrins dissolved in a removable liquid polyethylene glycol solution appears to be an effective method to improve the separation of the enantiomers of terbutaline by capillary electrophoresis. Complexation and therefore resolution increases when the cyclodextrin concentration is increased. The addition of polyethylene glycol also results in an increase in resolution. However at higher concentrations the baseline starts to fluctuate impairing quantitation of low concentrations of the individual enantiomers.

An other important variable is the temperature as we not only experienced with the CE experiments but also with the HPLC method as described in the experimental section. Baseline separation of racemic-terbutaline could only be achieved at relatively low temperatures.

Finally, impurity as low as 0.1% (distomer/eutomer) can be readily detected.

At the moment we are testing the method for other sympaticomimetic drug-selective β_2-receptor agonists and our goal is to design a model for optimal chiral separation that will be presented in a future paper.

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