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Separation of neutral dihydropyridines and their enantiomers using electrokinetic chromatography

Ann Van Eeckhaut**, Marc Robert Detaevernier, Yvette Michotte*

Department of Pharmaceutical Chemistry, Drug Analysis and Drug Information, Pharmaceutical Institute, Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Brussels, Belgium

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Abstract

The separation and simultaneous enantiomeric separation of three neutral 1,4-dihydropyridine (DHP) derivatives (nimodipine, nisoldipine and nitrendipine) was studied using electrokinetic chromatography. Bile salts allowed the non-chiral separation of these DHP derivatives. With the taurine-conjugated bile salts a beginning of enantiomeric separation was observed for nimodipine and nisoldipine. Achiral micelles of sodium dodecyl sulphate mixed with neutral cyclodextrins did not allow enantioseparation. Baseline chiral separation of nisoldipine and nimodipine was obtained with carboxymethyl-β-cyclodextrin at pH 5.0. The buffer type affected the chiral separation, especially in the case of nisoldipine. The addition of organic solvent decreased the enantioresolution of nimodipine. However, the resolution between the nisoldipine enantiomers was increased when methanol or ethanol were added to the background electrolyte. Varying the temperature had almost no effect on the enantioresolution of nisoldipine, whereas with nimodipine a clear improvement at lower temperatures was observed. Using the optimised method, the selectivity of this method was investigated for three possible impurities of nisoldipine.
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Keywords: Nimodipine; Nisoldipine; Nitrendipine; Electrokinetic chromatography; Chiral separation; Neutral dihydropyridines; Carboxymethyl-β-cyclodextrin

1. Introduction

Calcium channel blockers inhibit the entry of calcium ions via a subset of channels, leading to vasodilatation [1]. Therefore, they are effective in the treatment of essential hypertension and angina [2,3]. There are three main groups of calcium channel blockers of which 1,4-dihydropyridines (DHP) are an important class. The 1,4-DHP moiety is essential for their pharmacological activity on the cardiovascular system [4]. Except for nifedipine, all these drugs have a chiral carbon atom in position 4 of the dihydropyridine ring, due to the presence of asymmetric ester moieties. Most of these drugs are used as racemic mixture, but the pharmacological effects of the enantiomers can be different [1,2]. Therefore the development of stereoselective analysis methods is of great importance. For this purpose, capillary electrophoresis (CE) is still proving to be a highly effective tool [5–8].

Many chromatographic methods have already been developed for a wide variety of DHPs [1]. The chiral separation of these calcium antagonists with CE has also been described [3,9–12]. Gilar et al. [9] have studied the chiral separation of five DHP derivatives both with high performance liquid chromatography (LC) and capillary electromigration techniques.

Abbreviations: ACN, acetonitrile; BGE, background electrolyte; CyD, cyclodextrin; CE, capillary electrophoresis; CM-β-CyD, carboxymethyl-β-cyclodextrin; DM-β-CyD, heptakis(2,6-di-O-methyl)-β-cyclodextrin; DHP, dihydropyridine; EKC, electrokinetic chromatography; EOF, electroosmotic flow; EtOH, ethanol; HP-β-CyD, hydroxypropyl-β-cyclodextrin; HP-γ-CyD, hydroxypropyl-γ-cyclodextrin; IP, 2-propanol; LC, high performance liquid chromatography; MEKC, micellar electrokinetic chromatography; MeOH, methanol; MES, morpholinoethane sulfonic acid; SBE-β-CyD, sulfobutylether-β-cyclodextrin; SC, sodium cholate; SDC, sodium deoxycholate; SDS, sodium dodecyl sulphate; STC, sodium taurocholate; STDC, sodium taurodeoxycholate; TRIS, tris(hydroxymethyl)aminoethaan

* Corresponding author. Tel.: +32 2 4774429; fax: +32 2 4774113.
** Co-corresponding author. Present address: Faculty of Pharmacy, Free University Brussels, Laarbeeklaan 103, 1090 Brussels, Belgium.
E-mail addresses: aveeckha@vub.ac.be (A. Van Eeckhaut), ymichot@minf.vub.ac.be (Y. Michotte).

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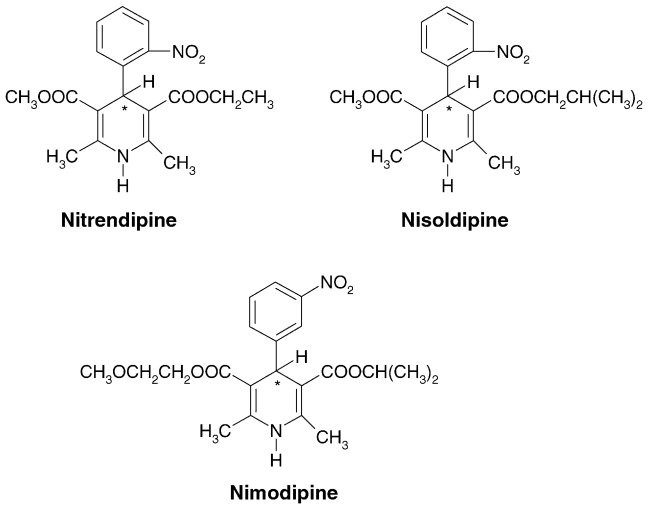

Fig. 1. Structure of nimodipine, nisoldipine and nitrendipine.

In the CE mode, carboxymethyl-β-CyD (CM-β-CyD) appeared to be the most suitable chiral selector. Baseline separation of nimodipine, nitrendipine and amlodipine enantiomers was achieved at pH 4.6. The separation of amlodipine enantiomers was also investigated by Owens et al. [10] using both neutral and anionic cyclodextrins (CyDs). Complete enantioseparation was obtained with hydroxypropyl-β-CyD (HP-β-CyD), CM-β-CyD and sulfobutylether-β-CyD (SBE-β-CyD). The enantiomers of amlodipine, nicardipine and four acidic DHP derivatives were separated with the native CyDs [3]. García-Ruiz and Marina [11] employed electrokinetic chromatography (EKC) to study the chiral separation of a large group of 1,4-DHP derivatives. The use of a 50 mM ammonium acetate buffer pH 6.7 and 25 mM CM-β-CyD, together with an applied voltage of 15 or 20 kV and a temperature of 15 °C enabled the individual enantiomeric separation of twelve DHP derivatives, although not all were baseline resolved. Christians et al. [12] separated 29 acidic, neutral and basic DHPs by means of neutral and negatively charged CyDs. They found that the neutral DHPs were baseline separated using SBE-β-CyD in the reversed polarity mode.

The three DHP derivatives tested in this work (nimodipine, nisoldipine and nitrendipine) are neutral molecules (Fig. 1). EKC has the capability to separate electrically neutral analytes. EKC combines the chromatographic separation principle with capillary electrophoretic techniques [11,13]. The different modes of EKC are all based on the differential partitioning of an analyte between a two-phase system: a mobile aqueous phase and a pseudostationary phase [14]. In micellar electrokinetic chromatography (MEKC), an ionic surfactant is added to the buffer at a concentration above the critical micellar concentration [15,16]. Two approaches can be used to perform enantiomeric separations in MEKC. Firstly, chiral surfactants (for example bile salts) have the ability to form micelles and to discriminate between enantiomers. Secondly, achiral surfactants can be used in combination with chiral additives like CyDs (CyD-MEKC) [7,16–19]. Another possibility for the enantioseparations of drug components is CyD-EKC, where charged CyDs are used as pseudostationary phases [20–22].

The aim of this study was to investigate these three possible EKC methods for the chiral separation of nimodipine, nisoldipine and nitrendipine, three neutral DHP derivatives. The influence of pH, buffer type, addition of organic modifier and temperature on the chiral separation of nimodipine and nisoldipine with CM-β-CyD as chiral selector was studied. Using the optimised method, its selectivity was investigated for three impurities of nisoldipine.

2. Experimental

2.1. Chemicals

Nimodipine, nisoldipine and nitrendipine were kindly provided by Bayer (Brussels, Belgium). Nisoldipine, its nitro- and nitrosopyridine compound were gifts from AstraZeneca (Brussels, Belgium). Nifedipine was obtained from Sigma Chemical Co. (St. Louis, MO, USA). All CyDs used were CE grade (purity >95%). Carboxymethyl-β-cyclodextrin (CM-β-CyD) (DS = 3 carboxymethyl groups/CyD ring) was purchased from Cyclolab (Budapest, Hungary). Heptakis(2,6-di-O-methyl)-β-cyclodextrin (DM-β-CyD), sodium cholate (SC), sodium taurocholate (STC), sodium deoxycholate (SDC), sodium taurodeoxycholate (STDC) and morpholinoethane sulfonic acid (MES) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Sodium tetraborate, boric acid, tris(hydroxymethyl)aminoethane (TRIS), orthophosphoric acid and sodium acetate were purchased from Merck (Darmstadt, Germany). Hydroxypropyl-β-cyclodextrin (HP-β-CyD) and hydroxypropyl-γ-cyclodextrin (HP-γ-CyD) were obtained from Aldrich (Gillingham, UK). Sodium dodecyl sulphate (SDS, electrophoresis purity grade) was obtained from BioRad (Richmond, CA, USA). Methanol, 2-propanol and acetontrile were obtained from Acros Organics (Geel, Belgium). Ethanol was purchased from Carlo Erba (Milan, Italy). All reagents were LC grade. The water used for preparing solutions was obtained from a Seralpur Pro 90 CN purification system (Seral, Germany).

2.2. CE equipment

A Beckman (Palo Alto, CA, USA) P/ACE 2100 System equipped with a UV detector and a temperature control system was used. All separations were performed in an uncoated fused silica capillary (Beckman) with a total length of 57 cm (50 cm to detector) × 75 μm i.d.. The CE instrument was controlled by the chromatography software System Gold 7.11 (Beckman). Online UV detection was performed at 254 nm. Unless stated otherwise, a voltage of 20 kV was applied and the capillary was temperature controlled at 25 °C by liquid cooling.

2.3. Conditions

Standard stock solutions of the analytes were prepared in methanol at a concentration of 1 mg/mL. Before injection the stock solution was diluted with the separation buffer to a concentration of 50 μg/mL. All solutions were protected from light. The sample solutions were injected in duplicate.

Sample solutions were introduced by pressure (0.5 psi) for 3 s or 5 s. Between runs, the capillary was flushed for 2 min with water and for 3 min with run buffer (20 psi). Resolution was calculated according to the following equation:

$$R_s = \frac{2(t_2 - t_1)}{w_1 + w_2}$$

where t_1 and t_2 are the migration times (min) of the two enantiomers and w_1 and w_2 are the corresponding peak widths, measured at baseline as the distance between the inflection tangents.

The preparation of the separation buffer depended on the mode of analysis. The different background electrolytes (BGEs) are described below.

2.3.1. MEKC with bile salts as chiral surfactants

The BGE pH 9.2 was prepared by dissolving appropriate amounts of sodium tetraborate in water and afterwards filtering it through a 0.2 μm membrane (Machery-Nagel, Düren, Germany). The appropriate amounts of SC or SDC were dissolved in the buffer solution and further diluted to volume with the same solution. The separation buffer for STC and STDC consisted of 0.05 M orthophosphoric acid adjusted to pH 5.0 with 1 M TRIS solution. The separation buffer was filtered and the appropriate amounts of bile salt were added.

2.3.2. CyD-MEKC

The separation buffer consisted of 0.05 M orthophosphoric acid adjusted to pH 7.0 with 1 M TRIS solution. The buffer was filtered and the appropriate amounts of SDS and CyD were added.

2.3.3. CyD-EKC

The separation buffer pH 9.2 was prepared as described in 2.3.1. For the separation electrolyte at pH 5.0 appropriate amounts of boric acid, sodium acetate or MES were dissolved in water, adjusted to pH 5.0 with 1 M sodium hydroxide solution and filtered. CM-β-CyD was added in the concentration required. In some cases organic modifier was added.

3. Results and discussion

3.1. MEKC with bile salts as chiral surfactants

Bile salts are naturally occurring anionic surfactants, which are structurally based on the cyclopentanephenantrene skeleton. Sodium cholate (SC) and sodium deoxycholate (SDC), non-conjugated bile salts, can be used in neutral and alkaline conditions. The taurine-conjugated forms, sodium taurocholate (STC) and sodium tauro-deoxycholate (STDC), can also be used in acidic conditions because of the presence of a sulfonic acid group in their structure [14,21,23,24]. In the experiments, the concentration of the buffer and bile salt and the applied voltage were varied.

In first instance, SC and SDC were tested as chiral agents. The best results were obtained with a BGE consisting of 30 mM sodium tetraborate pH 9.2 with 30 mM SC or 20 mM SDC and an applied voltage of 20 kV. In these systems, the three DHP compounds were separated from each other as racemates, although nimodipine and nisoldipine were not baseline resolved. They migrated in the following order: nitrendipine, nimodipine and nisoldipine. No chiral separation was observed. The addition of 10% methanol (MeOH) or acetonitrile (ACN) diminished or even completely abolished the separation.

With the taurine-conjugated bile salt, STDC, a beginning of enantiomeric separation was observed for nimodipine and nisoldipine, but complete chiral separation could not be attained. The same migration order as with the non-conjugated forms was observed. These experiments were carried out in a 50 mM phosphate buffer pH 5.0 containing 10 mM STDC. Higher concentrations of this surfactant could not be used, probably due to interferences of impurities. The results with STC are not clear due to interference of impurities. García-Ruiz and Marina [11] also studied the use of bile salts for the chiral separation of DHP derivatives. In their study they observed that only two out of eighteen derivatives were partially enantioseparated, which confirms our observations. An electropherogram of the non-chiral separation of nimodipine, nisoldipine and nitrendipine with SDC is shown in Fig. 2.

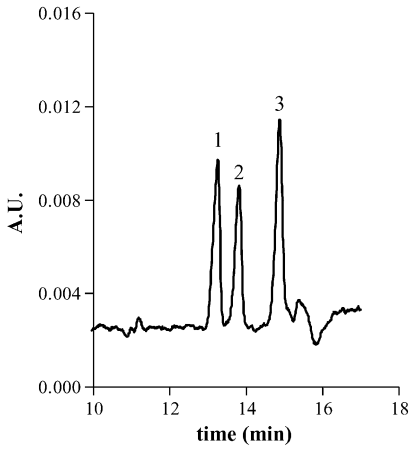

Fig. 2. Electropherogram of the non-chiral separation of nimodipine, nisoldipine and nitrendipine with MEKC using sodium deoxycholate (SDC) as chiral surfactant. Experimental conditions: capillary: 57 cm (50 cm effective length) × 75 μm i.d.; detection: 254 nm; applied voltage: 20 kV; temperature: 25 °C; injection: 3 s (pressure); separation solution: 30 mM sodium borate buffer pH 9.2 + 30 mM SDC. (1) Nitrendipine; (2) nimodipine; (3) nisoldipine.

3.2. CyD-MEKC

Besides using chiral surfactants, chiral separation of neutral racemates can also be performed by adding chiral additives, like CyDs, to a micellar solution. A neutral CyD is usually used with an achiral ionic surfactant, like sodium dodecyl sulphate (SDS), to achieve enantioseparation. The CyD cannot be incorporated into the micelles because of their hydrophilic surface and because the CyD is electrically neutral, it migrates with the velocity of the electro-osmotic flow (EOF). The surfactant molecule may, however, be included into the CyD cavity. Chiral resolution is therefore the result of the distribution of the analyte between three phases: the aqueous phase, the micel and the CyD [7,17,18,25].

The addition of 20 mM SDS to a 50 mM TRIS-phosphate buffer pH 7.0 enabled the separation of the tested DHP derivatives. The migration order was altered compared to the one obtained by addition of bile salts. Nisoldipine is now migrating before nimodipine. Three different CyD derivatives were added to the BGE in order to induce the enantioseparation of the compounds. However, the addition of 10 mM HP-β-CyD, HP-γ-CyD or DM-β-CyD did not result in chiral separation. The migration times of the three analytes were almost halved by the addition of the CyDs. This suggests that the analytes are less incorporated into the micelles in the presence of these CyD derivatives. However, no chiral recognition seems to occur. Both hydroxypropyl derivatives allowed the selective but non-chiral separation of the three racemic compounds, while nitrendipine and nimodipine co-migrated when DM-β-CyD was added to the BGE. The results of García-Ruiz and Marina [11] corroborated with our findings in the sence that no chiral separation using this separation mode was obtained. Fig. 3 shows the non-chiral separation of nimodipine, nitrendipine and nisoldipine using SDS in combination with HP-γ-CyD.

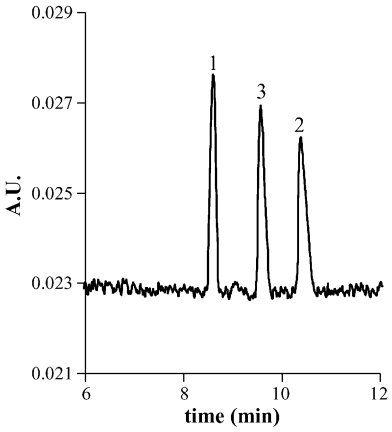

Fig. 3. Electropherogram of the non-chiral separation of nimodipine, nisoldipine and nitrendipine with CyD-MEKC. Experimental conditions: capillary: 57 cm (50 cm effective length) × 75 μm i.d.; detection: 254 nm; applied voltage: 30 kV; temperature: 25 °C; injection: 3 s (pressure); separation solution: 50 mM TRIS-phosphate buffer pH 7.0 + 20 mM SDS + 10 mM HP-γ-CyD; same numbering as in Fig. 2.

3.3. CyD-EKC

Charged CyD derivatives have their own mobility, which allows the separation of neutral compounds [22,26]. Separation is based on differential partitioning of solutes between the aqueous buffer and the charged CyD [18]. Ionisable CyDs often provide higher flexibility in the optimisation of enantiomeric resolution, mainly due to the possibility of changing their charge and hence, their electrophoretic mobility by altering the pH of the run buffer. Optimisation of separation conditions can easily be achieved by altering the CyD concentration or the running buffer pH. The introduction of charges on the CyD also enables additional electrostatic interactions between the analyte and the chiral selector [18,20,22,27].

Considering the good results with CM-β-CyD already mentioned in the literature [9–11], this chargeable CyD was selected for our experiments. At pH 9.2, the three compounds were baseline separated. The migration order was the same as with the bile salts. However, no chiral resolution of nitrendipine was observed and the enantiomers of nimodipine and nisoldipine were only partially resolved in this system. Complete chiral separation of nimodipine and nisoldipine was obtained when boric acid (pH 5.0) was used as BGE instead of sodium tetraborate (pH 9.2). The migration order did not change at this lower pH, where CM-β-CyD is still ionised. Increasing the pH of the boric acid BGE deteriorated the chiral separation. The combination of 10 mM CM-β-CyD with 10 mM of a neutral CyD, like β-CyD, HP-β-CyD or DM-β-CyD, deteriorated the chiral separation. Further investigations were performed with nimodipine and nisoldipine.

It is known that the buffer type can influence the chiral separation [28]. Three different buffer types were studied, namely boric acid, sodium acetate and MES, all at pH 5.0. The corresponding electropherograms are given in Fig. 4. The baseline chiral separation of both compounds was lost using the acetate buffer. The migration times of the enantiomers of nimodipine were approximately equal with boric acid (±30 min) or MES (±33 min) as BGE. For nisoldipine, a decrease in migration time was observed (±40 min versus ±30 min). This is also reflected in the enantioresolution that remained approximately equal for nimodipine (2.6 versus 2.7) and decreased for nisoldipine (2.9 versus 2.0). The type of buffer thus influenced the migration time and resolution of both DHP derivatives and especially affected nisoldipine. In further experiments the MES buffer was used. At pH 5.0 the EOF is also smaller which results in longer migration times. The application of a higher voltage (30 kV instead of 20 kV) solved this problem.

The addition of organic modifier can either improve or deteriorate the chiral separation. Organic modifiers can affect the binding constants of the enantiomer-CyD inclusion complexes as well as the EOF, the viscosity, the dielectric constant, the conductivity of the BGE, the solubility of either the analytes and/or the CyD [28–31]. The addition of MeOH, ethanol (EtOH), 2-propanol (IP) and ACN, in a concentration up to 15%, was investigated. These organic solvents

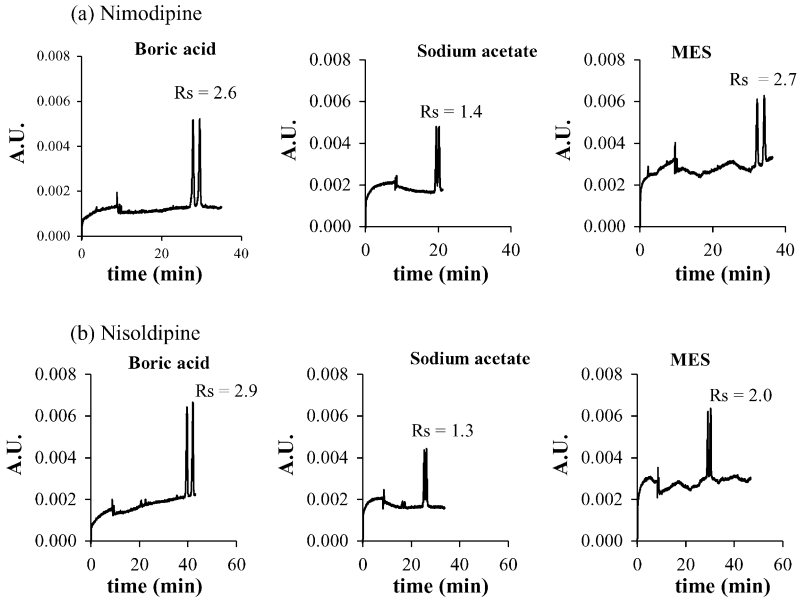

Fig. 4. Influence of the buffer type on the chiral separation of (a) nimodipine and (b) nisoldipine. Experimental conditions: capillary: 57 cm (50 cm effective length) × 75 μm i.d.; detection: 254 nm; applied voltage: 20 kV; temperature: 25 °C; injection: 5 s (pressure); separation solution: 60 mM buffer pH 5.0 + 15 mM CM-β-CD.

decreased the enantioresolution of nimodipine (Fig. 5a), while an increase is observed for nisoldipine as shown in Fig. 5b. For nisoldipine a continuous increase in resolution was observed with MeOH and EtOH, while the resolution decreased when higher concentrations than 10% ACN and 5% IP were used. From these results, it can be concluded that no common BGE could be obtained for an acceptable chiral separation of both compounds. For nimodipine, no organic solvent should be added, while for nisoldipine the highest resolution values were obtained with 15% MeOH.

The capillary temperature influences the mobility of the analytes, the kinetics and thermodynamics of the inclusion complexation process with CyDs [28]. An increase in temperature causes a decrease in BGE viscosity and thus an increase in mobility of the analytes. Furthermore, an increase in temperature generally causes a decrease in the binding constants [28,32]. The migration time of the enantiomers and their resolution decreased with increasing temperature. The temperature had almost no effect on the enantioresolution of nisoldipine. The resolution varied from 1.3 at 30 °C to 1.7 at 16 °C. However, the enantioresolution of nimodipine clearly improved at lower temperatures (from 1.2 at 30 °C to 2.5 at 16 °C).

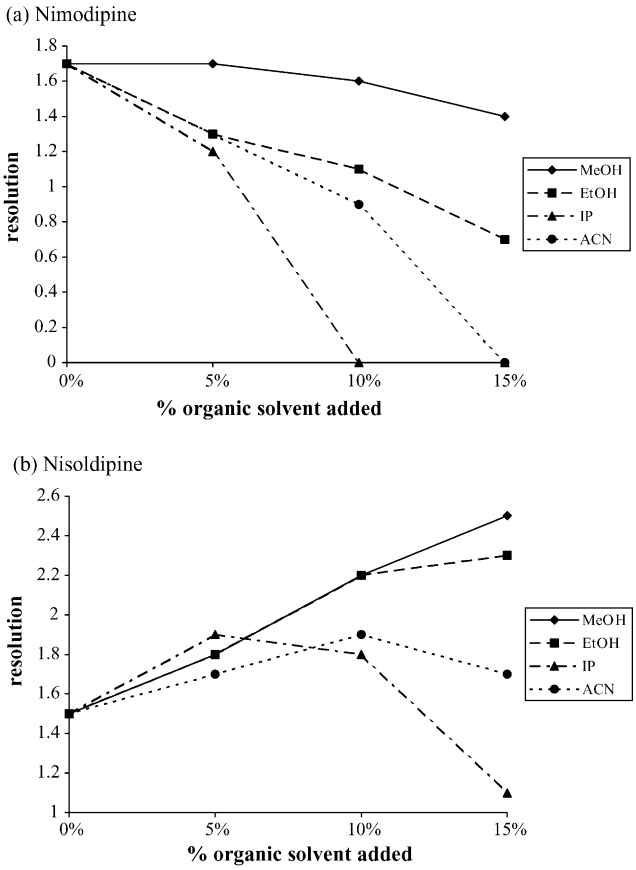

Fig. 5. Influence of the addition of organic solvent on the chiral separation of (a) nimodipine and (b) nisoldipine. Experimental conditions: see Fig. 4; applied voltage: 30 kV.

4. Optimised conditions

In Fig. 6, the electropherograms of the chiral separation of nisoldipine and nimodipine are shown, both obtained under their optimal conditions. As mentioned above, the optimal conditions were not completely the same for both compounds. The enantiomers of nimodipine were separated using

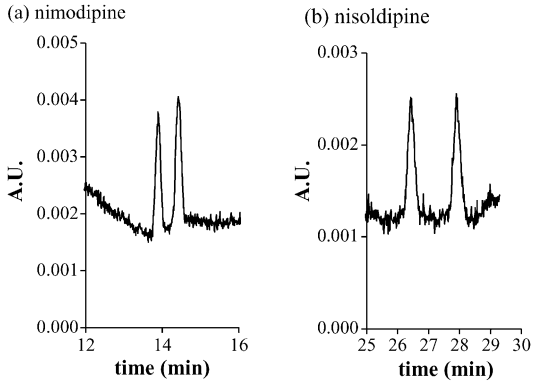

Fig. 6. Electropherograms of (a) nimodipine enantiomers and (b) nisoldipine enantiomers under optimised conditions. Experimental conditions: see Fig. 4; applied voltage: 30 kV; temperature: 16 °C; separation solution: (a) 60 mM MES buffer pH 5.0 + 15 mM CM-β-CyD, (b) 60 mM MES buffer pH 5.0 + 15 mM CM-β-CyD + 15% MeOH.

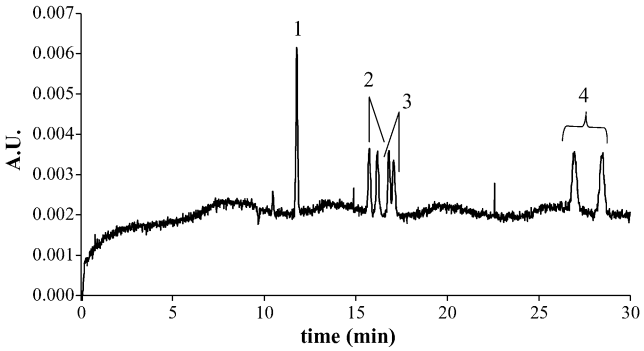

Fig. 7. Selectivity of the method for three impurities of nisoldipine: (1) nifedipine; (2) nitropyridine compound; (3) nitrosopyridine compound; (4) nisoldipine. Experimental conditions: capillary: 57 cm (50 cm effective length) × 75 μm i.d.; detection: 254 nm; applied voltage: 30 kV; temperature: 16 °C; injection: 5 s (pressure); separation solution: 60 mM MES buffer pH 5.0 + 15 mM CM-β-CyD + 15% MeOH.

a 60 mM MES buffer pH 5.0, containing 15 mM CM-β-CyD. For nisoldipine, 15% MeOH was added to this BGE.

Three possible impurities of nisoldipine (nifedipine, nitropyridine compound and nitrosopyridine compound) were injected under these optimised conditions for nisoldipine. Fig. 7 shows an electropherogram recorded with all four compounds. The three possible impurities were well separated from the nisoldipine peaks and the enantiomers of both pyridine compounds were also baseline resolved. However, the second migrating enantiomers of both pyridine compounds were not completely baseline separated, because the migration times of both peaks are similar.

5. Conclusions

Simultaneous achiral and chiral separation of neutral DHP derivatives is performed employing some EKC techniques. Baseline chiral separations for nimodipine and nisoldipine were only obtained in the CyD-EKC mode using CM-β-CyD as chiral selector. Optimising different parameters showed that no single system is adequate for the three derivatives. Baseline resolution of the enantiomers of nitrendipine could not be obtained in the tested systems. The chiral separation of nimodipine was almost not influenced by changing the BGE from boric acid to MES. However, this had a large effect on the chiral separation of nisoldipine. The addition of organic modifier decreased the enantioresolution of nimodipine. In the case of nisoldipine, an increase was observed when MeOH or EtOH were added. In contrast, varying the temperature had almost no effect on the enantioseparation of nisoldipine, but the chiral resolution of nimodipine was improved by decreasing the temperature. These results show that the influence of various experimental parameters on the chiral separation of closely related compounds can tremendously affect the outcome.

Complete chiral resolution of nimodipine is obtained using a 60 mM MES buffer pH 5.0 containing 15 mM CM-β-CyD, while for nisoldipine 15% MeOH was added. For the latter, the selectivity of the method was proven for three of its possible impurities.

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