AMINO ACID CHIRAL IONIC LIQUIDS COMBINED WITH HYDROXYPROPYL-β-CYCLODEXTRIN FOR DRUG ENANTIOSEPARATION BY CAPILLARY ELECTROPHORESIS

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Abstract

Four amino acid chiral ionic liquids were evaluated in dual systems with hydroxypropyl-β-cyclodextrin to investigate the enantioseparation by CE of a group of seven drugs as model compounds (duloxetine, verapamil, terbutaline, econazole, sulconazole, metoprolol, and nadolol). The use of two of these chiral ionic liquids ([TMA][L-Lys] and [TMA][L-Glu]) in CE is reported for the first time in this work whereas [TBA][L-Lys] and [TBA][L-Glu] were employed previously in CE although very scarcely. The effect of the nature and the concentration of each ionic liquid added to the separation buffer containing the neutral cyclodextrin on the enantiomeric resolution and the migration time obtained for each drug, was investigated. A synergistic effect was observed when combining each chiral ionic liquid with hydroxypropyl-β-cyclodextrin in the case of the five compounds for which the cyclodextrin showed enantiomeric discrimination power when used as sole chiral selector (duloxetine, verapamil, terbutaline, econazole, sulconazole). Buffer concentration and pH, temperature and separation voltage were varied in order to optimize the enantiomeric separation of these five compounds using dual systems giving rise to resolutions ranging from 1.1 to 6.6.

Keywords: Capillary electrophoresis, chiral ionic liquids, cyclodextrin, drugs, enantioseparation.

1. Introduction

The enantiomeric separation of chiral compounds has attracted great interest in the last years in the clinical, pharmaceutical or food fields, among others [1-4]. In particular, chirality is a relevant feature in the pharmaceutical field due to different reasons including the increasing trend to commercialize drugs as single enantiomers. Enantiomers have identical physicochemical properties in an achiral environment but different pharmacological, toxicological and biological activities. Often, one enantiomer produces the required therapeutic activity whereas the other could be inactive, can act in a different way or even be toxic. This fact highlights the relevance of the development of chiral methodologies.

Among the chromatographic and electrophoretic separation techniques most employed to achieve chiral separations (HPLC, GC, SFC, CE), CE is increasingly being used due to its interesting characteristics such as the possibility to obtain high efficiencies in short analysis times, low sample and reagent consumption, and the easy change of chiral selectors as they are added to the separation buffer in most cases (Electrokinetic Chromatography (EKC) separation mode). Among the different chiral selectors used in EKC, cyclodextrins (CDs) have been the most employed due to their high discrimination power [5]. However, the search for new chiral selectors to achieve enantiomeric separations by EKC or to improve the results obtained when combined with other chiral selectors in dual systems, presents a high interest. The possibility of using ionic liquids (ILs) in dual systems has shown to have a big potential [6].

ILs are organic molten salts constituted by bulky organic cations and organic or inorganic anions. They are called “designable solvents” [7] as there can be multiple combinations by varying the chemical composition of cations and anions, which influences their unique

properties such as high conductivity, negligible vapor pressure, good thermal stability and miscibility in different solvents. This feature has allowed the use of these materials in different areas like electrochemical reactions, organic and inorganic synthesis or analytical chemistry [8, 9].

There are ILs which have a chiral moiety on their structure and that are called chiral ionic liquids (CILs). Although there are some works devoted to the use of CILs as sole chiral selectors in CE, most studies reported the use of dual systems formed by CILs and CDs, polysaccharides, cyclofructans or surfactants [10]. In general, these dual systems provided better chiral separations in comparison with the use of single systems, showing a synergistic effect between both kinds of chiral selectors.

Regarding the synthesis of CILs, one possibility is to synthesize them from low cost chiral sources such as amino acids, which can constitute the cationic or the anionic part of the CILs since they have an amino group and a carboxylic moiety. Several studies have been carried out using amino acid based ILs (AAILs) as chiral selectors. In particular, those formed by amino acid anions have been investigated in combination with different selectors as polysaccharides [11, 12] and antibiotics [13] for the separation of chiral drugs, in combination with CDs [14-17] for the enantioseparation of amino acids, alkaloids and drugs, and also as ligands in ligand-exchange CE (LE-CE) [18] for the separation of amino acids.

The aim of this work was to evaluate four CILs (tetramethylammonium L-Lysine ([TMA][L-Lys]), tetrabutylammonium L-lysine ([TBA][L-Lys]), tetramethylammonium L-glutamic acid ([TMA][L-Glu]), and tetrabutylammonium L-glutamic acid ([TBA][L-Glu])) to investigate the enantioseparation by EKC of seven drugs as model compounds when dual systems of each CIL with the neutral CD hydroxypropyl-β-CD (HP-β-CD)

were employed as chiral selectors in the separation buffer. From these four CILs, [TBA][L-Lys] and [TBA][L-Glu] were previously used in CE in combination with β-CD for the enantioseparation of phenethylamines [19] and the use of [TBA][L-Glu] in combination with HP-β-CD was reported for the stereoselective recognition of corynoxine [20]. However, the use of [TMA][L-Lys] and [TMA][L-Glu] in CE is reported for the first time in this work.

2. Materials and methods

2.1. *Reagents and samples*

Reagents and chemicals of analytical grade have been used to perform all experiments. Ortho-phosphoric acid, methanol, ethanol, Na_2SO_4 and acetonitrile (ACN) were provided by Scharlau (Barcelona, Spain). Sodium hydroxide, α-cyclodextrin (α-CD), methyl-β-cyclodextrin (M-β-CD, DS~10.5-14.7), heptakis(2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD), heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (TM-β-CD), carboxymethyl-β-cyclodextrin (CM-β-CD, DS~3) were obtained from Sigma-Aldrich (Saint-Louis, MO, USA). β-cyclodextrin (β-CD), γ-cyclodextrin (γ-CD), and 2-hydroxypropyl-β-cyclodextrin (HP-β-CD, DS~0.6) were purchased from Fluka (Buchs, Switzerland), whereas acetyl-β-cyclodextrin (Ac-β-CD, DS~7) and succinyl-β-cyclodextrin (Suc-β-CD, DS~3.5) were from CycloLab (Budapest, Hungary). Water employed to prepare all solutions was obtained with a Milli-Q system from Millipore (Bedford, MA, USA).

L-glutamic acid, L-Lysine, tetramethylammonium hydroxide (TMAOH) pentahydrate, and tetrabutylammonium hydroxide (TBAOH) 30-hydrate purchased from Sigma Aldrich (Saint-Louis, MO, USA) were employed to carry out the synthesis of the different CILs.

Terbutaline (2-t-butylamino-1-[3,5-dihydroxyphenyl]ethanol hemisulfate salt), verapamil (5-[N-(3,4-dimethoxyphenylethyl)methylamino]-2-(3,4-dimethoxyphenyl)-2-isopropylvaleronitrile hydrochloride), econazole (1-(2-[(4-chlorophenyl)methoxy]-2-[2,4-dichlorophenyl]ethyl)-1H-imidazole nitrate salt) and sulconazole (1-(2-[p-chlorobenzylthio]-2-[2,4-dichlorophenyl]ethyl)-1H-imidazole nitrate salt) were obtained from Sigma-Aldrich (Saint-Louis, MO, USA) as racemic mixtures. Nadolol ((2R,3S)-5-[3-(tert-butylamino)-2-hydroxypropoxy]-1,2,3,4-tetrahydronaphthalene-2,3-diol) and metopropol (1-[4-(2-metoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-2-propanol tartrate) were also provided as racemic mixtures by J. Uriach & Cia, S.A. (Barcelona, Spain) and Astra Hässle AB (Mölndal, Sweden), respectively. Duloxetine, both racemic mixture and pure enantiomer (N-Methyl-3-(1-naphthalenyloxy)-3-(2-thrienyl)propanamine hydrochloride) were provided by IS Chemical Technology (Shanghai, China). The structures of the drugs analyzed in this work are shown in **Figure 1A.**

2.2. *Synthesis of the chiral ionic liquids*

The four CILs used in this work, [TMA][L-Lys], [TBA][L-Lys], [TMA][L-Glu], and [TBA][L-Glu] (see **Figure 1B**), were synthesized by the Center for Applied Chemistry and Biotechnology (CQAB) from the University of Alcalá using as starting materials the commercially available L-amino acids (used as received without extra purification) and exploiting simple acid-based chemistry [21, 22]. The two CILs with L-Glu as amino acid were synthesized in their monosubstituted forms. In general, [TMA][L-Lys] and [TMA][L-Glu] were synthesized adding a 40% aqueous solution of TMAOH pentahydrate (1.0 eq.) to an aqueous suspension of the desired amino acid (1.0 eq.). The

resulting reaction mixture was heated at 60 ºC for 2 h, and the water was removed under reduced pressure. Then, the resultant residue was dissolved in ethanol and filtered to remove the unreacted amino acid. The filtrate was dried over Na_2SO_4, filtrated, and the solvent was removed under vacuum to afford the desired product. Every product was dried under high vacuum (50 mbar) and 45 ºC until constant weight was observed. [TBA][L-Lys] and [TBA][L-Glu] were prepared mixing an aqueous solution of TBAOH 30-hydrate (98%) (1,0 eq.) to an aqueous suspension of the desired amino acid (1.0 eq.) and following the procedure described previously for TMA ionic liquids, but in this case the resultant residue was dissolved in ACN instead of ethanol. All the CILs were characterized by HPLC-UV-MS (MS) and ^{1}H proton nuclear magnetic resonance (^{1}H-NMR).

2.3. *CE conditions*

This work was carried out with a 7100 CE system from Agilent Technologies (Palo Alto, CA, USA) equipped with a diode array detector (DAD). The CE system was controlled by the Agilent ChemStation software. Separations were performed using uncoated fused-silica capillaries of 50 µm ID (362 µm OD) with an effective length of 50 cm (total length of 58.5 cm). Injections were made by applying a pressure of 50 mbar for 4 s and the detection wavelength was set at 200 nm for each drug, except for duloxetine (220 nm), with a bandwidth of 4 nm. Analysis were performed using a voltage of 20 or 30 kV at a working temperature of 15 or 20ºC.

New capillaries were conditioned (applying 1 bar) with NaOH 1M (30 min), Milli-Q water (15 min) and with running buffer (1 h). At the beginning of each working day, the capillary was rinsed with NaOH 0.1M for 5 min, Milli-Q water for 5 min and 30 min of

running buffer. Between injections, the capillary was conditioned with NaOH 0.1M (3 min), Milli-Q water (2 min) and background electrolyte (BGE) during 8 min when the BGE contained CILs or mixtures CILs/HP-β-CD. In case of using a BGE containing only CDs, the capillary was rinsed for 3 min.

2.4. *Preparation of solutions and samples*

Buffer solutions were prepared by diluting the appropriate volume of ortho-phosphoric acid in Milli-Q water to obtain the desired concentration and adjusting the pH to 2.5 or 3.0 with NaOH 1 M. BGEs containing CD were prepared dissolving the adequate amount of each CD in 50 or 100 mM phosphate buffer to obtain different concentrations. BGEs containing CILs or mixtures of CILs and HP-β-CD were prepared dissolving the appropriate amount of these compounds in the buffer solution and adjusting the pH to 2.5 or 3.0 with ortho-phosphoric acid.

Stock standard solutions of duloxetine, nadolol, metoprolol, verapamil, and terbutaline were prepared dissolving the adequate amount of each drug in Milli-Q water to obtain a final concentration of 1000 mg L^{-1}, whereas to obtain the same concentration of econazole and sulconazole standards, they were dissolved in methanol. All these standard solutions were stored at 4ºC. From them, solutions of 150 mg L^{-1} of each drug were used in method optimization, which were prepared by diluting an appropriate aliquot in Milli-Q water.

All working solutions were filtered through 0.45 µm Nylon syringe filters (Scharlau, Barcelona, Spain) and sonicated before analysis.

2.5. *Data treatment*

The ChemStation software from Agilent Technologies was used to obtain the resolution values between adjacent peaks. Origin 8.0 software was employed to the composition of figures with different electropherograms.

3. Results and discussion

In order to study the effect of using the chiral AAILs synthesized in this work ([TMA][L-Lys], [TBA][L-Lys], [TMA][L-Glu], and [TBA][L-Glu]) combined with a CD in a dual system in CE, the enantiomeric separation of seven model drugs (duloxetine, verapamil, terbutaline, econazole, sulconazole, metoprolol, and nadolol) was investigated. A screening of neutral CDs was carried out to choose the most adequate CD to achieve this study. Ten CDs were assayed including the three native CDs (α-CD, β-CD, and γ-CD) and other seven derivatives (M-β-CD, DM-β-CD, HP-β-CD, TM-β-CD, CM-β-CD, Suc-β-CD, and Ac-β-CD). Each CD was added at a 5 mM concentration using as preliminary experimental conditions a 100 mM phosphate buffer (pH 2.5), a temperature of 20 ºC and a voltage of 20 kV. Under these acidic conditions, the seven drugs were positively charged. HP-β-CD was chosen as chiral selector since it enabled the chiral discrimination of five out of the seven drugs studied (duloxetine, verapamil, terbutaline, econazole, sulconazole) whereas the other CDs originated chiral discrimination for two (α-CD, γ-CD, and TM-β-CD), three (M-β-CD, DM-β-CD, Suc-β-CD, and Ac-β-CD) or four drugs (β-CD and CM-β-CD) (see **Table 1**). The five drugs enantiomerically separated when a 5 mM concentration of HP-β-CD was assayed, were also separated when a 2 mM concentration of this CD was employed in the separation buffer although in some cases resolutions obtained were lower than 1.0 at one or both CD concentrations. Enantiomeric resolutions and migrations times for the five drugs for which enantiomeric discrimination

was observed with HP-β-CD as sole chiral selector in the separation buffer (at a concentration of 2 and 5 mM) are shown in **Table 2**. No enantioseparation was observed for any of the model compounds analyzed when a AAILs was used alone as chiral selector in the separation buffer.

3.1. *Effect of the nature of the chiral ionic liquid*

The four CILs were individually added at three concentrations (5, 10 and 30 mM) to the separation buffer containing 2 mM or 5 mM HP-β-CD. Enantiomeric resolutions and migrations times obtained for the drugs analyzed are shown in **Table 2**. It can be observed that a synergistic effect occurred when adding the four CILs in the case of duloxetine, verapamil, terbutaline, econazole and sulconazole that were the compounds that showed enantiomeric separation when using 2 or 5 mM HP-β-CD alone in the separation buffer. No chiral discrimination was observed under these conditions for nadolol and metoprolol that were not enantiomerically separated when using HP-β-CD as sole chiral selector at any of the two concentrations assayed. The CIL [TBA][L-Lys] provided the highest values for the enantiomeric resolution in the case of duloxetine (Rs 7.5) and terbutaline (Rs 5.9) followed by the ionic liquid [TBA][L-Glu] (Rs for duloxetine 6.9, Rs for terbutaline 5.4) being the enantiomeric resolutions originated by these two ILs the highest in the case of econazole and sulconazole with values similar for both ILs (Rs 5.2 for econazole and Rs close to 2.0 for sulconazole, with both ILs). Finally, [TMA][L-Glu] provided slightly higher values for the enantiomeric resolution in the case of verapamil (Rs 1.4) than those obtained with [TBA][L-Lys] and [TBA][L-Glu] (Rs 1.3). Although the values obtained for the enantiomeric resolution for verapamil using the dual system of HP-β-CD with the ILs investigated were lower than those obtained for the other

compounds, the use of these CILs supposed an increment of around twofold in the enantiomeric resolution obtained when using HP-β-CD alone in the separation buffer. The results obtained showed that [TBA][L-Lys] and [TBA][L-Glu] can be considered the CILs providing the best enantiomeric resolutions for the model drugs analyzed as a consequence of a synergistic effect when used in combination with HP-β-CD in a dual system.

3.2. *Effect of the concentration of the chiral ionic liquids*

Table 2 shows that, as general trend, an increase in the enantiomeric resolution can be observed when increasing the concentration of the ionic liquid. This is true for all ILs and both concentrations of HP-β-CD assayed (2 and 5 mM) although in most cases the highest increases in the enantiomeric resolution values were observed at a 5 mM concentration of HP-β-CD. Exception to this behavior could be econazole since in this case the highest enantiomeric resolutions were not obtained always with 5 mM HP-β-CD (an enantiomeric resolution of 5.1 was also obtained with 2 mM HP-β-CD and 30 mM [TMA][L-Lys]). In the case of sulconazole, the effect of using a 30 mM concentration of CILs could not be evaluated due the long analysis times obtained under these conditions (analyses were stopped at 60 min). Taking into account these results, a 30 mM concentration of [TBA][L-Lys] and [TBA][L-Glu] with a concentration of 5 mM HP-β-CD can be considered the conditions under which a strongest synergistic effect could be observed for duloxetine, terbutaline, verapamil and econazole. In the case of sulconazole, better enantiomeric resolutions were obtained for 2 mM HP-β-CD with respect to those observed for CD concentrations of 5 mM.

3.3. *Effect of the buffer concentration and pH*

The effect of decreasing the buffer concentration from 100 to 50 mM when maintaining a pH value of 2.5 was studied for the combination of 30 mM [TBA][L-Lys] with 2 or 5 mM HP-β-CD (**Table 3**). Results showed that a decrease in the enantiomeric resolution was observed for duloxetine and terbutaline at 5 mM HP-β-CD when decreasing buffer concentration. In the case of verapamil, similar values of the enantiomeric resolution were obtained when decreasing the buffer concentration whereas analysis times higher than 60 min were observed for econazole and sulconazole. Although for 2 mM HP-β-CD, slightly higher values of enantiomeric resolution could be observed for verapamil, terbutaline and econazole when decreasing the buffer concentration, longer analysis times were generally obtained. Consequently, a 100 mM buffer concentration was selected in order to investigate the effect of increasing the buffer pH.

Under the conditions that originated the strongest synergistic effect for duloxetine, verapamil, terbutaline and econazole (30 mM [TBA][L-Lys] or [TBA][L-Glu] and 5 mM HP-β-CD), an increase in the buffer pH from 2.5 to 3.0 lead to a decrease in the enantiomeric resolution for duloxetine and a slightly increase in this parameter for verapamil regardless the ionic liquid employed. Analysis times higher than 60 min were also obtained for sulconazole at pH 3.0 for both CILs. Different variations were observed when increasing the pH for the other drugs depending on the ionic liquid nature (see **Table 4**). In fact, the enantiomeric resolution for terbutaline decreased for [TBA][L-Lys] and increased for [TBA][L-Glu] when increasing the pH to 3.0 while the contrary was observed for econazole illustrating the importance of the ionic liquid nature when studying the effect of the buffer pH and enabling to choose a pH value of 2.5 for further

experiments since a clear improvement in the enantiomeric resolution was not generally observed at pH 3.0 for the drugs studied.

3.4. *Effect of the temperature and applied voltage*

The effect of decreasing the temperature from 20ºC to 15ºC was investigated for a buffer concentration of 100 mM (pH 2.5) when using 30 mM [TBA][L-Lys] or [TBA][L-Glu] and 5 mM HP-β-CD. As it can be observed in **Table 4**, higher enantiomeric resolutions were obtained for duloxetine, verapamil, terbutaline and econazole at 15 ºC regardless the ionic liquid used, confirming the favorable effect of decreasing the temperature on the chiral recognition in dual systems as observed when using single chiral selectors as CDs [23, 24]. As expected, an increase in the migration times was observed due to the increase in the solution viscosity when decreasing the working temperature. Using a temperature of 15ºC, an increase of the separation voltage from 20 to 30 kV enabled to decrease the analysis times although a decrease was also observed for the enantiomeric resolutions, especially for terbutaline and econazole when using [TBA][L-Lys], as shown in **Table 4**. Under these conditions, enantiomeric resolutions close to 1.9 and 1.8 were obtained for sulconazole for the first time using 30 mM [TBA][L-Lys] or [TBA][L-Glu], respectively.

Since a 2 mM concentration of HP-β-CD originated the highest enantiomeric resolutions for sulconazole (see section 3.2), this CD concentration was employed at 15 ºC and 30 kV. The results obtained showed that, under these conditions, enantiomeric resolutions of 2.7 (27.0 min) and 2.8 (28.0 min) were achieved for sulconazole with [TBA][L-Lys] and [TBA][L-Glu], respectively, being these the most favourable conditions for this drug. **Figure 2** shows the enantiomeric separations for the five compounds analyzed under the

optimized conditions for the dual systems selected and when the CD was employed as the sole chiral selector in the background electrolyte.

4. Conclusions

The combined use of the four CILs evaluated in this work ([TMA][L-Lys], [TBA][L-Lys], [TMA][L-Glu]) with HP-β-CD enabled the enantiomeric separation by CE of five (duloxetine, verapamil, terbutaline, econazole and sulconazole) out of seven model drugs investigated. This is the first time that [TMA][L-Lys] and [TMA][L-Glu] are synthesized and evaluated as chiral selectors in CE. A synergistic effect was observed when using combinations of the four CILs and HP-β-CD (at concentrations of 2 or 5 mM). Investigation of the effect of the nature and concentration of the CILs on the enantiomeric separation of the compounds studied revealed that the strongest synergistic effect for duloxetine, verapamil, terbutaline and econazole occurred when using 30 mM [TBA][L-Lys] or [TBA][L-Glu] and 5 mM HP-β-CD while the best results for sulconazole were obtained for 2 mM HP-β-CD. For other drugs (metoprolol and nadolol) that were not enantiomerically separated with HP-β-CD when used as sole chiral selector, no synergistic effect was observed when adding any of the CILs to the separation buffer at the concentrations of CDs assayed. A buffer concentration of 100 mM, a buffer pH of 2.5, and a temperature of 15 ºC enabled to obtain the highest enantiomeric resolutions for the drug studied with the selected dual chiral systems. The application of 30 kV as separation voltage considerably decreased analysis times with assumable decreasing in the enantiomeric resolutions. The results obtained in this work show that the CILs studied have potential as synergistic systems to improve the enantiomeric resolutions for drugs when using low concentrations of CDs as chiral selector.

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Figure captions.

Figure 1. Structures of the drugs (A) and the CILs (B) studied in this work.

Figure 2. Electropherograms corresponding to the enantioseparation of five chiral drugs using HP-β-CD as single chiral selector or dual systems based on mixtures of HP-β-CD and [TBA][L-Glu] or [TBA][L-Lys]. CE conditions: 100 mM phosphate buffer (pH 2.5); uncoated fused-silica capillary, 50 μM ID x 58.5 cm (50 cm of effective length); UV detection at 200 nm (220 nm for duloxetine); temperature 15ºC; applied voltage, 30 kV; injection by pressure, 50 mbar for 4 s.

Table 1. Enantiomeric resolutions and migration times for seven chiral drugs with ten different neutral CDs.

CD	Duloxetine*		Metoprolol		Nadolol		Verapamil		Terbutaline		Econazole		Sulconazole	
	Rs	t1	Rs	t1	Rs	t1	Rs	t1	Rs	t1	Rs	t1	Rs	t1
α-CD	NS	12.8	NS	19.8	NS	17.3	NS	16.2	NS	13.5	0.6	27.2	0.7	26.5
β-CD	0.6	20.6	NS	18.3	NS	14.7	0.9	22.3	2.3	15.1	1.6	21.9	NS	23.7
γ-CD	0.9	17.0	NS	14.3	NS	14.5	NS	17.1	NS	13.1	1.5	21.7	NS	25.0
HP-β-CD	3.3	22.6	NS	18.1	NS	15.1	0.6	21.5	3.5	15.3	3.1	22.8	0.6	25.9
M-β-CD	1.1	22.3	NS	16.9	NS	14.7	NS	23.5	4.6	15.9	0.4	22.9	NS	24.6
DM-β-CD	0.9	27.4	0.7	18.5	NS	15.2	NS	29.5	5.3	21.1	NS	26.3	NS	26.6
TM-β-CD	NS	15.2	NS	13.7	NS	14.2	1.5	16.5	NS	13.4	NS	16.8	1.0	18.9
CM-β-CD	-	> 60	NS	21.3	NS	15.6	3.8	46.9	4.1	22.5	2.0	41.6	1.0	43.4
Suc-β-CD	2.5	25.1	NS	18.6	NS	14.9	1.6	24.0	NS	17.0	1.2	26.1	NS	27.9
Ac-β-CD	1.0	24.1	NS	19.1	NS	14.6	1.6	24.4	2.6	19.4	NS	24.8	NS	25.1

CE conditions: 100 mM phosphate buffer (pH 2.5) with 5 mM CD; uncoated fused-silica capillary, 50 µM ID x 58.5 cm (50 cm of effective length); UV detection at 200 nm (220 nm for duloxetine); temperature 20ºC; applied voltage, 20 kV; injection by pressure, 50 mbar for 4 s.
Rs: Resolution; t1: time of the first-migrating enantiomer (min).
*Analyses were stopped at 60 min and Rs values were not determined.

Table 2. Enantiomeric resolutions and migration times for the chiral drugs by CE.

Chiral drug	Duloxetine		Verapamil		Terbutaline		Econazole		Sulconazole*	
	Rs	t1	Rs	t1	Rs	t1	Rs	t1	Rs	t1
HP-β-CD 2 mM	3.9	18.2	0.5	18.8	2.7	14.3	3.4	19.2	1.6	22.6
+ 5 mM [TMA][L-Lys]	3.8	19.2	0.6	21.8	2.2	14.9	4.3	22.5	1.9	28.2
+ 10 mM [TMA][L-Lys]	4.0	22.9	0.6	26.5	2.4	17.3	3.7	27.4	1.8	36.6
+ 30 mM [TMA][L-Lys]	5.0	34.2	1.0	38.4	2.7	23.2	5.1	40.6	-	> 60
+ 5 mM [TBA][L-Lys]	3.8	22.5	0.6	23.9	2.3	16.3	3.5	24.7	1.8	31.4
+ 10 mM [TBA][L-Lys]	4.1	24.6	0.6	26.6	2.4	17.6	3.9	27.5	2.0	36.2
+ 30 mM [TBA][L-Lys]	5.6	30.8	0.8	35.9	2.8	23.7	5.1	38.9	-	> 60
+ 5 mM [TMA][L-Glu]	3.5	20.6	0.5	21.3	1.9	15.5	3.2	21.6	1.6	28.4
+ 10 mM [TMA][L-Glu]	3.6	24.2	0.6	27.1	2.2	18.1	3.3	28.0	1.8	36.6
+ 30 mM [TMA][L-Glu]	5.0	37.5	0.8	36.5	2.5	22.0	4.3	37.4	-	> 60
+ 5 mM [TBA][L-Glu]	3.7	21.7	0.5	23.1	2.3	16.4	3.1	23.6	1.6	30.2
+ 10 mM [TBA][L-Glu]	3.8	23.8	0.6	25.7	2.4	17.9	3.6	26.4	2.1	35.0
+ 30 mM [TBA][L-Glu]	5.4	30.0	0.8	35.3	3.0	23.6	4.7	38.2	-	> 60
HP-β-CD 5 mM	3.6	18.9	0.7	21.3	3.7	15.5	3.1	22.5	0.7	24.3
+ 5 mM [TMA][L-Lys]	4.1	24.4	1.0	27.8	4.2	16.8	3.6	30.0	1.0	33.4
+ 10 mM [TMA][L-Lys]	4.4	29.4	0.9	31.9	4.5	19.0	3.8	34.1	1.0	40.2
+ 30 mM [TMA][L-Lys]	6.2	48.3	1.3	49.7	4.9	26.1	4.5	55.3	-	> 60
+ 5 mM [TBA][L-Lys]	4.1	26.0	0.8	25.9	3.9	18.3	3.1	28.2	1.0	33.7
+ 10 mM [TBA][L-Lys]	4.3	29.6	0.9	29.8	4.0	19.5	3.4	32.4	1.1	40.3
+ 30 mM [TBA][L-Lys]	7.5	50.6	1.3	46.6	5.9	26.7	5.2	56.5	-	> 60
+ 5 mM [TMA][L-Glu]	3.5	24.7	0.8	27.6	3.7	18.1	2.9	29.1	1.0	33.8
+ 10 mM [TMA][L-Glu]	3.6	27.7	1.0	37.9	4.0	21.3	2.9	36.0	0.9	41.7
+ 30 mM [TMA][L-Glu]	6.4	53.3	1.4	53.6	4.8	27.1	4.6	62.4	-	> 60
+ 5 mM [TBA][L-Glu]	3.9	26.2	0.7	26.3	3.9	17.9	2.5	28.6	0.9	32.9
+ 10 mM [TBA][L-Glu]	4.1	29.8	0.9	30.8	4.3	19.8	3.6	33.6	1.0	40.1
+ 30 mM [TBA][L-Glu]	6.9	42.2	1.3	44.0	5.4	26.1	5.2	51.7	-	> 60

CE conditions: 100 mM phosphate buffer (pH 2.5); uncoated fused-silica capillary, 50 µM ID x 58.5 cm (50 cm of effective length); UV detection at 200 nm (220 nm for duloxetine); temperature 20ºC; applied voltage, 20 kV; injection by pressure, 50 mbar for 4 s.
Rs: Resolution; t1: time of the first-migrating enantiomer (min). *Analyses were stopped at 60 min and Rs values were not determined.

Table 3. Enantiomeric resolutions and migration times for the chiral drugs by CE at different buffer concentrations.

Chiral selectors	Phosphate buffer	Duloxetine		Verapamil		Terbutaline		Econazole		Sulconazole*	
30 mM [TBA][L-Lys]		Rs	t1	Rs	t1	Rs	t1	Rs	t1	Rs	t1
+ 2 mM HP-β-CD	100 mM	5.6	30.8	0.8	35.9	2.8	23.7	5.1	38.9	-	> 60
	50 mM	5.5	32.4	0.9	38.4	3.1	24.6	5.4	42.1	-	> 60
+ 5 mM HP-β-CD	100 mM	7.5	50.6	1.3	46.6	5.9	26.7	5.2	56.5	-	> 60
	50 mM	7.1	47.8	1.2	51.0	5.7	28.1	-	> 60	-	> 60

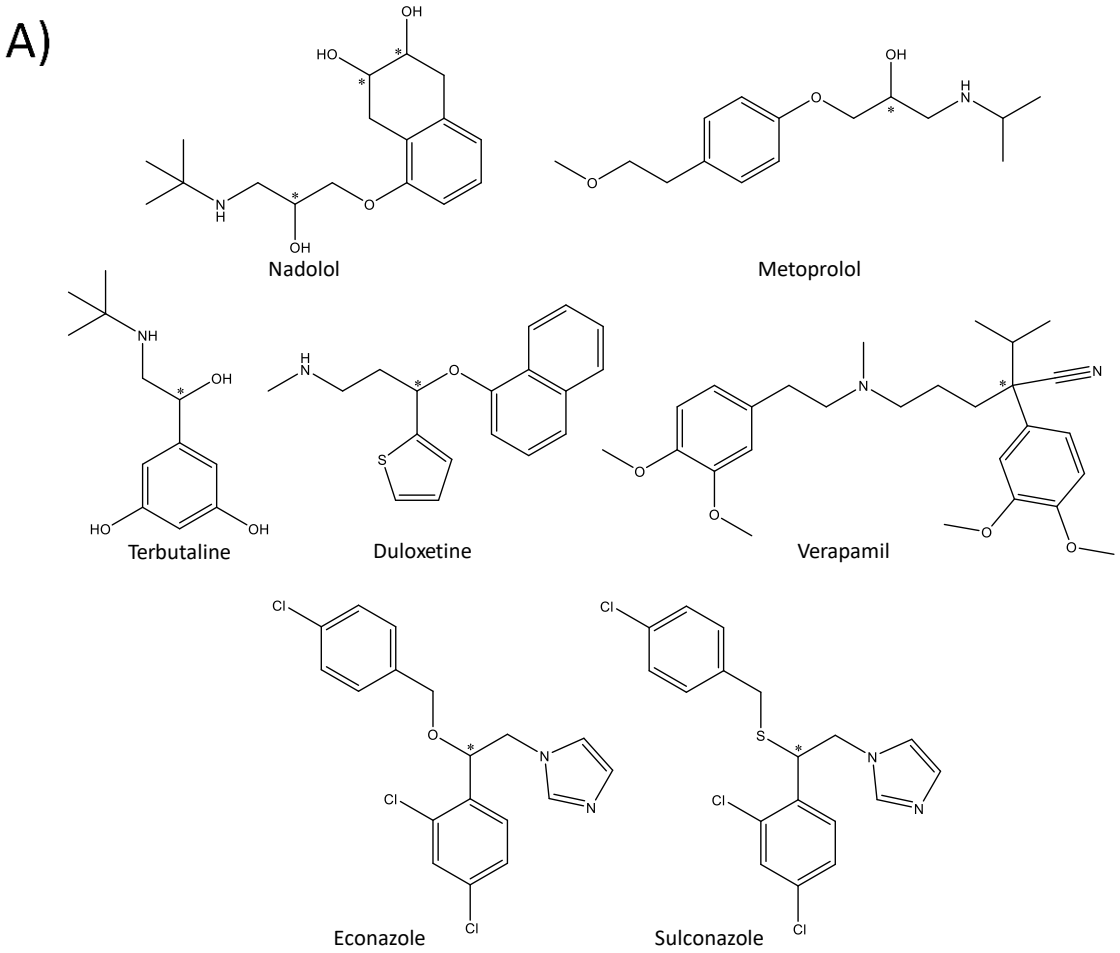
CE conditions: phosphate buffer (pH 2.5); uncoated fused-silica capillary, 50 µM ID x 58.5 cm (50 cm of effective length); UV detection at 200 nm (220 nm for duloxetine); temperature 20ºC; applied voltage, 20 kV; injection by pressure, 50 mbar for 4 s.
Rs: Resolution; t1: time of the first-migrating enantiomer (min). *Analyses were stopped at 60 min and Rs values were not determined.

Table 4. Enantiomeric resolutions and migration times for the chiral drugs by CE at different working temperatures and separation voltages.

Chiral selectors	pH	Temperature (ºC)	Voltage (kV)	Duloxetine		Verapamil		Terbutaline		Econazole		Sulconazole*	
5 mM HP-β-CD				Rs	t1	Rs	t1	Rs	t1	Rs	t1	Rs	t1
+ 30 mM [TBA][L-Lys]	3.0	20	20	6.8	45.1	1.4	48.7	5.5	27.6	5.4	58.2	-	> 60
	2.5	20	20	7.5	50.6	1.3	46.6	5.9	26.7	5.2	56.5	-	> 60
	2.5	15	20	7.6	52.0	1.4	54.5	6.2	30.9	6.0	62.8	-	> 60
	2.5	15	30	6.3	20.3	1.1	21.5	4.6	16.8	4.1	24.7	1.9	31.2
+ 30 mM [TBA][L-Glu]	3.0	20	20	6.3	42.0	1.4	45.1	6.0	26.0	5.0	50.6	-	> 60
	2.5	20	20	6.9	42.2	1.3	44.0	5.4	26.1	5.2	51.7	-	> 60
	2.5	15	20	7.1	47.9	1.3	50.3	6.3	29.4	5.6	57.7	-	> 60
	2.5	15	30	6.6	21.8	1.1	23.1	3.9	14.0	4.3	26.2	1.8	33.3

CE conditions: 100 mM phosphate buffer; uncoated fused-silica capillary, 50 µM ID x 58.5 cm (50 cm of effective length); UV detection at 200 nm (220 nm for duloxetine); injection by pressure, 50 mbar for 4 s.
Rs: Resolution; t1: time of the first-migrating enantiomer (min). *Analyses were stopped at 60 min and Rs values were not determined.

Figure 1.


A)
Nadolol
Metoprolol
Terbutaline
Duloxetine
Verapamil
Econazole
Sulconazole

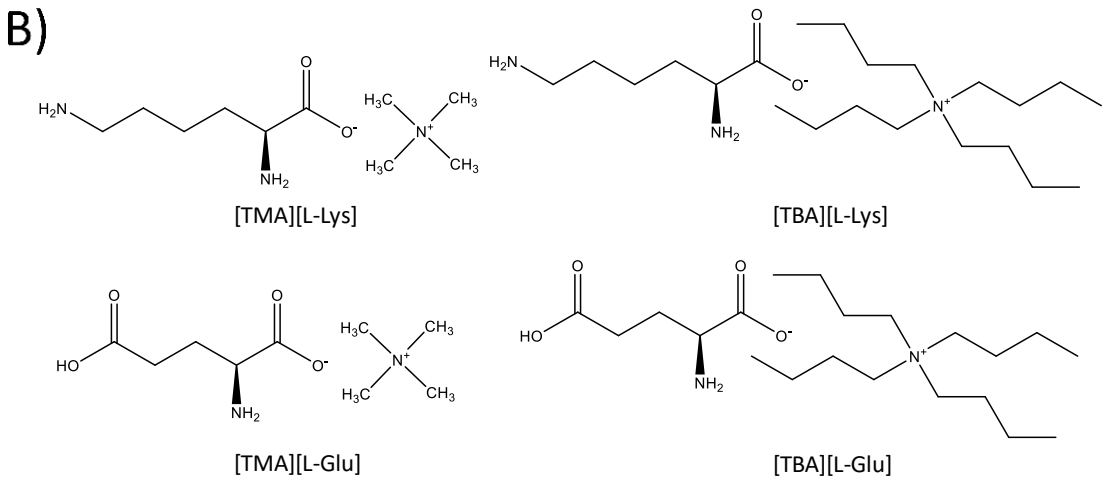
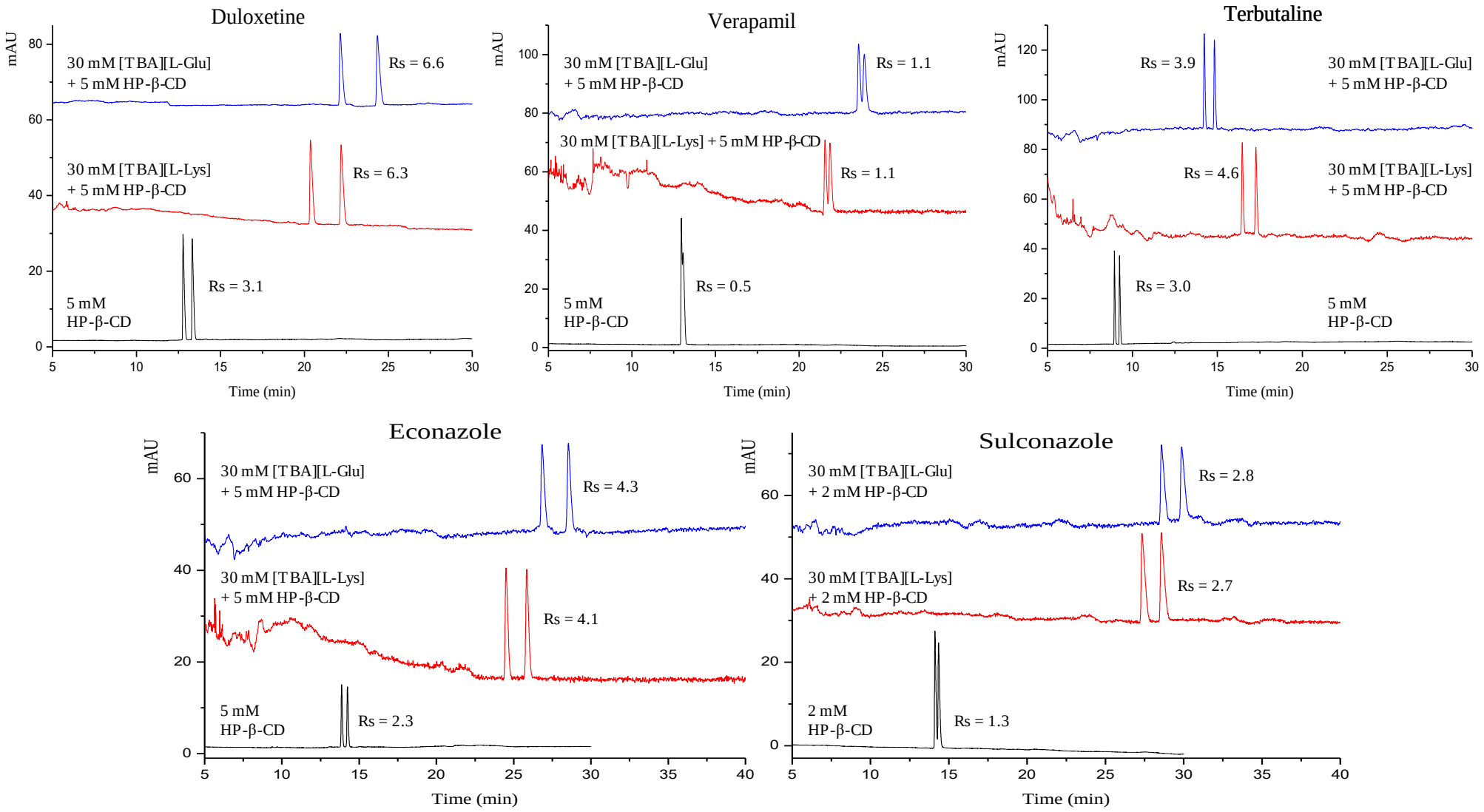

B)
[TMA][L-Lys]
[TBA][L-Lys]
[TMA][L-Glu]
[TBA][L-Glu]

Figure 2.


Duloxetine
mAU
30 mM [TBA][L-Glu]
+ 5 mM HP-β-CD
Rs = 6.6
30 mM [TBA][L-Lys]
+ 5 mM HP-β-CD
Rs = 6.3
5 mM
HP-β-CD
Rs = 3.1
Time (min)
Verapamil
30 mM [TBA][L-Glu]
+ 5 mM HP-β-CD
Rs = 1.1
30 mM [TBA][L-Lys] + 5 mM HP-β-CD
Rs = 1.1
5 mM
HP-β-CD
Rs = 0.5
Time (min)
Terbutaline
Rs = 3.9
30 mM [TBA][L-Glu]
+ 5 mM HP-β-CD
Rs = 4.6
30 mM [TBA][L-Lys]
+ 5 mM HP-β-CD
Rs = 3.0
5 mM
HP-β-CD
Time (min)
Econazole
30 mM [TBA][L-Glu]
+ 5 mM HP-β-CD
Rs = 4.3
30 mM [TBA][L-Lys]
+ 5 mM HP-β-CD
Rs = 4.1
5 mM
HP-β-CD
Rs = 2.3
Time (min)
Sulconazole
30 mM [TBA][L-Glu]
+ 2 mM HP-β-CD
Rs = 2.8
30 mM [TBA][L-Lys]
+ 2 mM HP-β-CD
Rs = 2.7
2 mM
HP-β-CD
Rs = 1.3
Time (min)