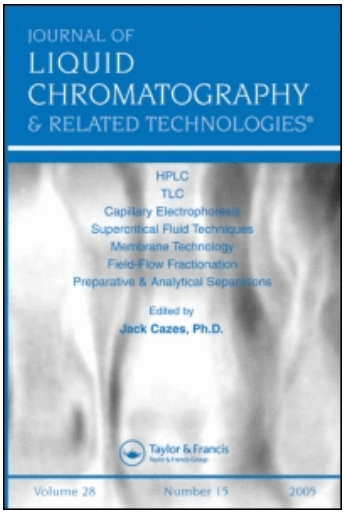

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CHIRAL CAPILLARY ELECTROPHORETIC SEPARATION OF SELECTED DRUGS AND METABOLITES USING SULFATED β-CYCLODEXTRIN

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ABSTRACT

In this paper, we report the chiral separation of some drugs and their metabolites using sulfated β-cyclodextrin, a powerful chiral selector that has proven to be highly efficient for the separation of chiral drugs, alone or in combination, with other chiral additives. Praziquantel, its metabolite trans-4-hydroxypraziquantel, and albendazole sulfoxide are neutral compounds and could be separated at basic pH (8–10).

The simultaneous chiral separation of praziquantel and its metabolite enantiomers was only possible by the addition of sodium deoxycholate to the running buffer. Fluoxetine, disopyramide, and mexiletine, as well as their metabolites are basic com-

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pounds and were separated by appropriate selection of the running buffer pH and CD concentration. In addition, the effects of the experimental parameters, such as concentration of sulfated β-cyclodextrin, pH and buffer concentration, temperature, and voltage were also evaluated.

Among the several parameters studied, the concentration of the chiral selector and the pH were the most important to obtain the chiral separation of the selected drugs and metabolites.

INTRODUCTION

Twenty five percent of all drugs used as therapeutic agents are chiral compounds administered to humans as a racemate, a mixture of enantiomers which may have very different pharmacological properties. This fact led the Food and Drug Administration (FDA) in the USA and similar regulatory agencies in Europe and Japan, to establish guidelines which recommend the production of chiral drugs as single enantiomers.[1–3] This new trend resulted in an increase in the demand for enantioselective methods for the analysis of drugs in raw material, pharmaceutical formulations, and biological fluids, in order to check the enantiomeric purity and to permit pharmacokinetic and metabolic studies.[4]

High performance liquid chromatography using chiral stationary phases, was the preferred technique for this purpose until the advent of capillary electrophoresis (CE) in the last decade. The main advantages of CE for the enantioselective analysis of chiral drugs are the extremely high efficiency, instrumentation simplicity, low sample and reagent consumption, and speed in method development and analysis. In addition, CE is a complementary technique to high performance liquid chromatography, particularly for the analysis of charged and polar compounds.[5–7]

Although chiral CE separations can be accomplished in different modes, capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) are the most widely used methods,[6,8,9] corresponding to 72% and 21% of all original articles published in the field, respectively.[10] The separation of enantiomers by CZE and MEKC is possible by the addition of chiral selectors to the running buffer. The most common chiral selectors are cyclodextrins and their derivatives, due to the commercial availability of various kind of these selectors, relatively low cost, and their UV transparency, stability, and solubility in aqueous buffers.[10,11] According to Blaschke and Chankvetadze,[12] recent literature reports (97–99) show that 85% of chiral CE separations for biomedical purposes were performed using cyclodextrins (CD).

Among several charged and non charged CD derivatives currently in use, a new charged cyclodextrin derivative, sulfated β-cyclodextrin (sulfated β-CD) has

proven to be highly efficient for the separation of chiral drugs. Due to its negative charge, sulfated β-CD has its own electrophoretic mobility, which also allows its use for the separation of neutral or ionizable compounds in uncharged form.

The enantiomeric separation using sulfated β-CD is based on the formation of diastereomeric complexes due to the drug inclusion in the CD cavity. The complexation constants are primarily determined by the size, geometry, hydrophobicity, and hydrogen-bonding properties of the analytes. In addition to these interactions, the electrostatic interaction of charged analytes with the negatively charged functional group of the CD can also occur.[11,13,14]

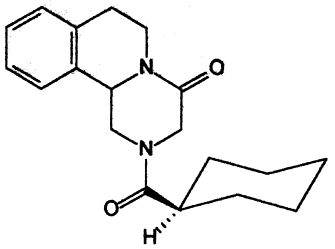
During the last few years, several papers have appeared in the literature reporting the separation of chiral drugs using sulfated β-CD as chiral selector under aqueous[13–18] or non-aqueous conditions.[19,20] Most of these papers deal with the separation of the drugs and not with the simultaneous resolution of the drugs and their metabolites. However, in order to use these procedures for drug analysis in biological samples it is important to separate not only the parent drug but also the chiral metabolites that could be found in the samples, mainly when these metabolites contribute to the pharmacological properties of the drug. Since the minor differences introduced in the chemical structures of metabolites during metabolism may lead to very different electrophoretic and stereoselective behavior, the separation conditions should be optimized for both parent drug and metabolites.[21]

On this basis, we report, here, the chiral separation of some drugs and their metabolites using sulfated β-CD. The optimized conditions allowed the simultaneous analysis of the drugs and their metabolites in a single run. In addition, we performed a systematic study evaluating the influence of several parameters on chiral separation. The structures of the drugs and metabolites selected for the present study are shown in Figure 1. Except for fluoxetine[22,23] and praziquantel,[24] the simultaneous chiral separation of the parent drug and metabolites has not been previously reported.

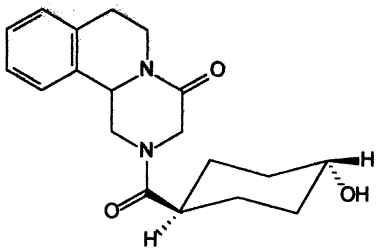
EXPERIMENTAL

Instrumentation

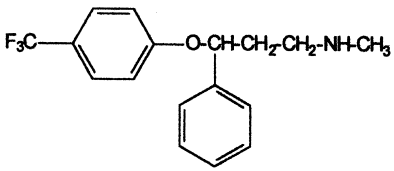
All experiments were carried out using a capillary electrophoresis instrument, CE ULTRA, TermoQuest Co. (San Jose, CA, USA), equipped with an autosampler device, temperature control, and a UV-VIS detector operating at certain wavelengths according to the drug and metabolite analyzed. The separation was performed in uncoated fused-silica capillaries (Eberline Instruments, Santa Fe, USA), whose internal diameter and length are specified in Table 1. Samples were hydrodynamically introduced into the capillary. Other operating parameters are also described in Table 1.



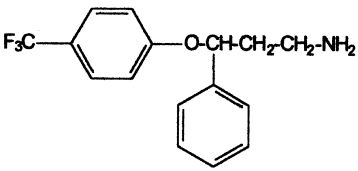
Praziquantel



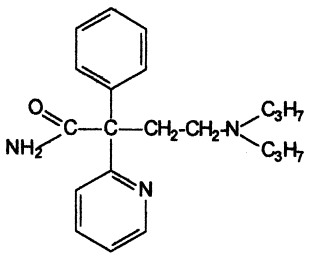
Trans-4-hydroxypraziquantel



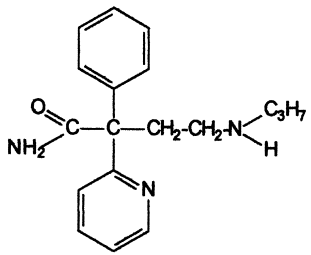
Fluoxetine



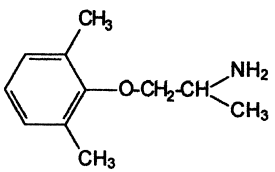
Norfluoxetine



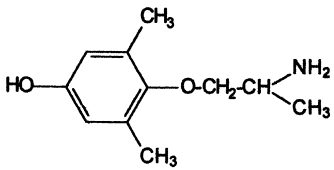
Disopyramide



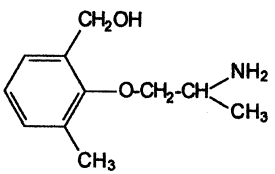
Mono-N-dealkyldisopyramide



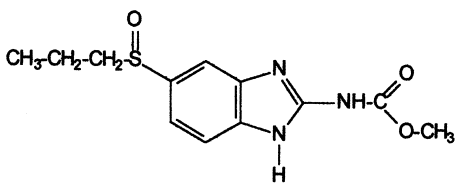
Mexiletine



p-Hydroxymexiletine



Hydroxymethylmexiletine



Albendazole Sulfoxide

Figure 1. Chemical structures of the drugs and metabolites.

Table 1. Electrophoretic Separation Conditions

	Praziquantel/ Metabolite	Albendazole Sulfoxide	Disopyramide/ Metabolite	Fluoxetine/ Metabolite	Mexiletine/ Metabolites
Capillary dimensions	50 μm × 66 cm (60 cm)*	50 μm × 66 cm (60 cm)*	75 μm × 42 cm (36 cm)*	75 μm × 42 cm (36 cm)*	75 μm × 42 cm (36 cm)*
Plain buffer	Borate 20 mmol/L, pH 10.0	Borate 20 mmol/L, pH 9.4	Phosphate 20 mmol/L, pH 5.0	Borate 20 mmol/L, pH 10,0	Phosphate 10 mmol/L, pH 7.0
Sulfated β-CD concentration	1 %	3%	0.8 %	0.5 %	2 %
Buffer additive (20 mm)	Sodium deoxycholate	Sodium deoxycholate	-	-	-
Temperature (°C)	20	15	20	25	20
Voltage (KV)	15	15	15	12	10
Wavelength detection (nm)	212	290	263	220	220
Solvent for the sample	Urea 4 mol/L	Urea 4 mol/L	Running buffer 10 fold diluted	Running buffer 10 fold diluted	Plain buffer
Injection conditions	0.8 psi × 2.5 s	0.5 psi × 2.5 s	0.8 psi × 5 s	0.8 psi × 5 s	0.8 psi × 5 s

* Effective capillary length.

Chemicals, Drugs, and Metabolites

The chiral selectors, sulfated β-CD (degree of substitution = 7–11), and sodium deoxycholate were purchased from Supelco (Bellefonte, PA, USA) and TermoQuest Co. (San Jose, CA, USA), respectively. All other chemicals were P.A. grade and were provided by Merck (Darmstadt, Germany).

The racemic drugs and metabolites were kindly supplied by the following pharmaceutical industries: Robert Young & Co. Ltd., Glasgow, Scotland (albendazole sulfoxide); Merck, Darmstadt, Germany (praziquantel); Boehringer Ingelheim, Ingelheim, Germany (mexiletine and its metabolites); Laboratórios Silva Araújo Roussel - Roussel UCLAF, Rio de Janeiro, Brazil (disopyramide and its metabolite); Eli Lilly do Brasil Ltda, São Paulo, SP, Brazil (fluoxetine). Norfluoxetine was purchased from Research Biochemicals, Inc. (Natick, MA, USA) and trans-4-hidroxypraziquantel was a generous gift from Dr. G. Blaschke (University of Münster, Münster, Germany).

Solutions

The plain buffer solutions were prepared in water purified with a MilliQ-plus system (Millipore Co., Bedford, USA) at the desired concentration and pH and the chiral selector was further added to obtain the final concentration. The solutions were then sonicated for 5 min and filtered through 0.45 μm membranes (Millipore Co., Bedford, USA).

Capillary Preparation

New capillaries were conditioned by rinsing with water (0.4 min, 30°C), NaOH 1 mol/L (0.4 min, 30°C and 0.4 min, 60°C), NaOH 0.1 mol/L (0.4 min, 30°C and 0.4 min, 60°C) and water (0.4 min, 30°C).

Before each analysis the capillaries were washed with NaOH 0.1 mol/L (1 min), water (1 min) and the running buffer (1 min). These washing procedures were carried out at 100 p.s.i and at the temperature used for the analysis.

Procedure

Drug and metabolite solutions were prepared in methanol at the concentration of 100 μg/mL. Immediately before the analysis 25 μL of these solutions were transferred to clean tubes and the solvent was evaporated under an air flow. The

residues were dissolved as specified in Table 1. An aliquot of these solutions was transferred to micro vials and analyzed.

The electrophoretic parameters were calculated using the following equations: $\mu_A = lL/tV$, where μ_A is the apparent solute mobility, l is the effective capillary length, L is the total capillary length, t is the migration time and V is the applied voltage; $\alpha = \mu_{A1} / \mu_{A2}$, where α is the enantioselective factor and μ_{A1} and μ_{A2} are the apparent mobility for the first and second enantiomer, respectively; $R = 2(t_2 - t_1)/ (w_1 + w_2)$, where R is the resolution factor, t_2 and t_1 are the migration time for the second and first enantiomer and w_1 and w_2 are the respective baseline peak width.

RESULTS AND DISCUSSION

In the present study, we report the chiral separation of some drugs and their metabolites using sulfated β-CD. The optimized conditions for the simultaneous analysis of each drug and metabolites are described in Table 1, and the electropherograms obtained are shown in Figures 2–6. A normal voltage was applied in all experiments (injection at the anode and detection at the cathode).

The enantiomers of praziquantel and trans-4-hydroxypraziquantel could be separated under basic conditions (Figure 2). These neutral compounds show no electrophoretic mobility and their migration depends on the electrosmotic flow (EOF), which does not differentiate the enantiomers. The separation of the enantiomers was obtained by enantioselective complexation with sulfated β-CD, which has a counter-EOF migration pattern. Although sulfated β-CD is highly effective for the chiral separation of praziquantel and its metabolite, it does not differentiate the parent drug from its metabolite because the metabolization site is far from the chiral center (Figure 1). The simultaneous chiral separation of the parent drug and its metabolite was obtained by the addition of sodium deoxycholate to the running buffer.

Lerch and Blaschke[24] have reported the enantioselective analysis of praziquantel, trans-4-hydroxypraziquantel, and other hydroxy metabolites using sulfobutyl ether β-CD at pH 5.25. According to these investigators, for the simultaneous separation of the drug and its metabolites, a concentration of 4 mmol/L of the chiral selector was required. In our case, the concentration of sulfated β-CD was increased up to 15 mmol/L without any improvement in the chiral separation of the parent drug and its metabolite.

Albendazole sulfoxide is the chiral and active metabolite of albendazole, the drug used for the treatment of neurocysticercosis, which is not a chiral compound. Due to its extensive metabolism, albendazole is not detected in plasma samples, and therefore, pharmacokinetic studies are carried out by quantification of the active metabolite.[25] The chiral separation of albendazole sulfoxide was

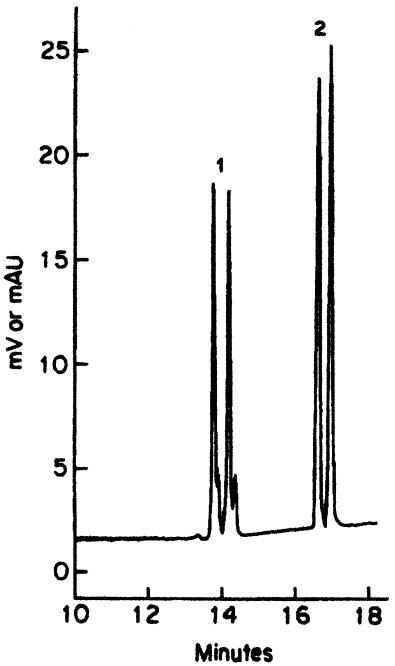


Figure 2. Electropherogram for the chiral separation of trans-4-hydroxypraziquantel (1) and praziquantel (2) using sulfated β-CD. Conditions described in Table 1.

obtained under conditions similar to those used for praziquantel and its metabolite, except for the higher CD concentration (Figure 3). Although albendazole sulfoxide enantiomers could be separated using only sulfated β-CD as chiral selector, better separation was obtained with a mixture of both sulfated β-CD and sodium deoxycholate.

The other drugs and metabolites studied were basic compounds and have their own electrophoretic mobility, so the separation of their enantiomers is possible using neutral or charged CD derivatives. The chiral separation of disopyramide and/or mexiletine has been described using sulfobutyl ether β–CD,[26–28] dimethyl β-CD,[16,27,29,30] trimethyl β-CD,[27,30] hydroxypropyl β-CD,[27] phosphated γ-CD,[31] and sulfated β-CD,[14,17] although none of these papers described the separation of the metabolites. Fluoxetine enantiomers have also been separated using trimethyl β-CD,[32] hydroxypropyl β-CD, dimethyl β-CD, and sulfated β-CD.[16] In a recent paper, Desiderio et al.[23] used a mixture of a neutral and a negatively charged CD, dimethyl β-CD and phosphated γ-CD, respectively, for the separation of both fluoxetine and norfluoxetine enantiomers. The chiral separation of

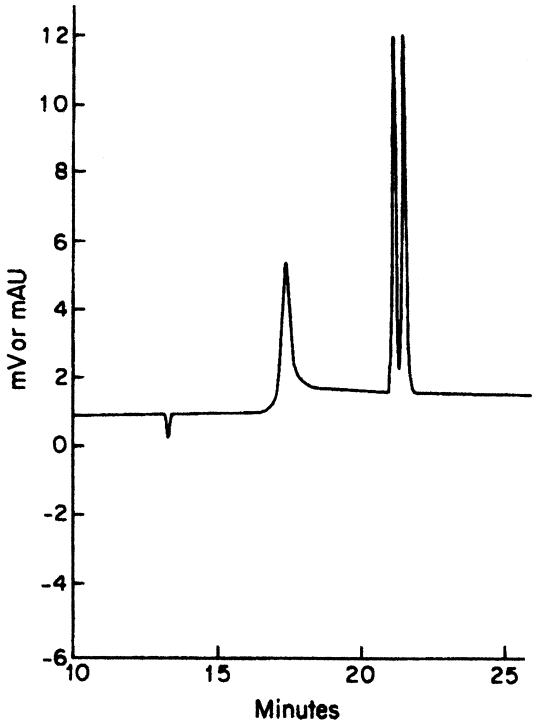


Figure 3. Electropherogram for the chiral separation of albendazole sulfoxide using sulfated β-CD. Conditions described in Table 1.

fluoxetine and norfluoxetine was also reported by Piperaki et al.[22] using sulfobutyl ether β-CD.

Figures 4–6 show the chiral separation of these drugs and their metabolites using sulfated β-CD, and Table 1 shows the electrophoretic conditions. In general, better separation was obtained than reported in the literature.

The analysis of mexiletine, fluoxetine, and their metabolites using sulfated β-CD resulted in peak tailing. This behavior has also been observed for basic drugs using this chiral selector[17,20] and phosphated γ-CD,[23] and was explained as a consequence of adsorption of the analyte to the inner capillary wall or formation of several inclusion complexes and electrodispersion phenomena due to the presence of several cyclodextrin isomers (the sulfated β-CD used in the present study was a mixture of randomly substituted CD).

The solution for this problem was evaluated for fluoxetine and its metabolite. Our first assumption was that the tail was due to the interaction with silanol groups in the inner wall of the capillary. The addition of poly vinyl alcohol (0.1 to

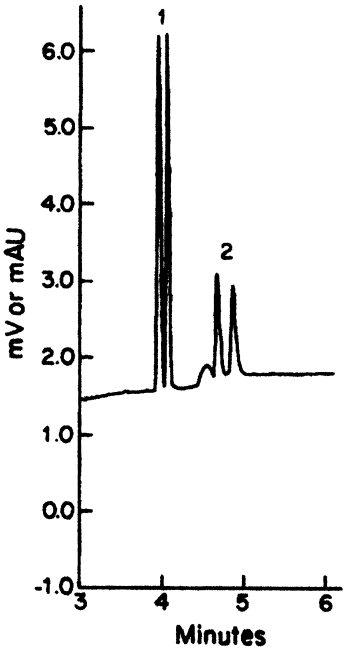


Figure 4. Electropherogram for the chiral separation of disopyramide (1) and mono-N-dealkyldisopyramide (2) using sulfated β-CD. Conditions described in Table 1.

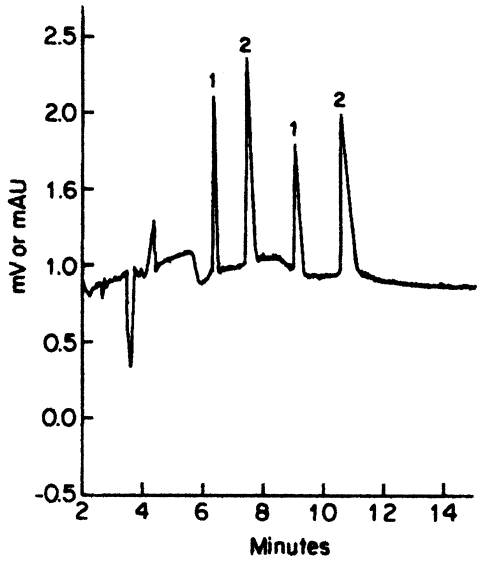


Figure 5. Electropherogram for the chiral separation of norfluoxetine (1) and fluoxetine (2) using sulfated β-CD. Conditions described in Table 1.

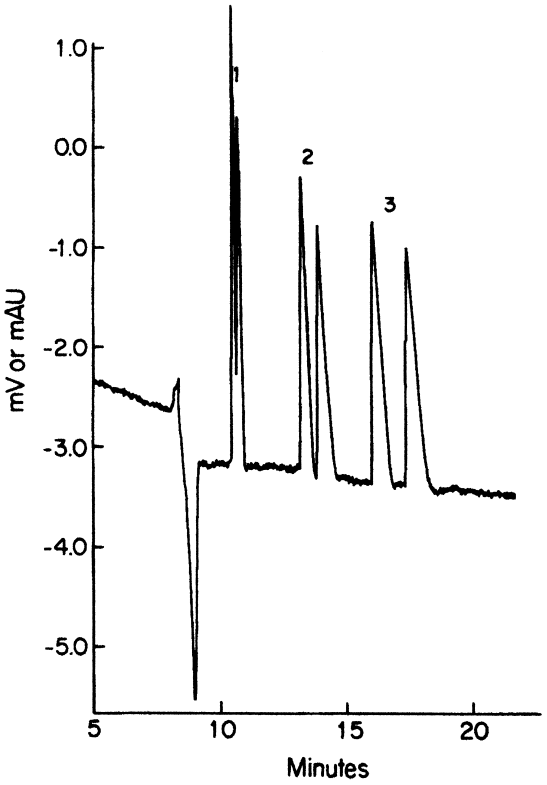


Figure 6. Electropherogram for the chiral separation of hydroxymethylmexiletine (1), mexiletine (2) and p-hydroxymexiletine (3) using sulfated β-CD. Conditions described in Table 1.

0.5 %) to the running buffer, or the increase in the running buffer pH to 11.5, did not improve the symmetry of the peaks. Based on this, we concluded that the tailing observed for the basic compounds was due to the interaction between the randomly substituted charged CD and the analytes. Under these circumstances, electrodispersion is the main reason for peak tailing.

Several authors have used reversed polarity (injection at the cathode and detection at the anode side) for the analysis of basic compounds using sulfated β-CD and buffer pH around 2–3.[14,15,17] This assumption was also evaluated for fluoxetine and its metabolite but did not reduce the peak tail. In addition, it resulted in longer analysis times and the requirement of higher CD concentration to obtain the chiral separation of the drugs.

Influence of CD Concentration

CD concentration is one of the most important parameters for the improvement of separation. The optimum concentration depends on the affinity constants of both enantiomers with the chiral selector, which means that both the kind of compound being separated and the type of CD used define the optimum concentration.[6] At a low CD concentration, no separation of the enantiomers is possible because there is not enough chiral selector available to form the complexes.

When CD concentration is increased, enantioselective complexation occurs, which allows the chiral separation of the analytes. At the high concentration extreme, both enantiomers are completely complexed and no more separation is observed. Other parameters, such as running buffer pH, which affect the enantiomer-CD interaction, also affect the optimum concentration of the chiral selector.[6,8]

The apparent mobility of the enantiomers for all drugs and metabolites studied, decreased with the increase in sulfated β-CD concentration, indicating a greater complexation with the chiral selector. It should also be pointed out, that when the sulfated β-CD is increased, the ionic strength also increases with a consequent reduction in the EOF. The enantioseparation (α) also increased for all drugs in the concentration range studied, except for disopyramide and its metabolite for which a optimum concentration, depending on the drug, was observed (Table 2).

Table 2. Influence of Sulfated β-CD Concentration

Drug / Metabolite	CD (%, w/v)	μ_{A1} ($\times 10^{-4}$)*	μ_{A2} ($\times 10^{-4}$)*	α*	R*
Disopyramide	0.13	5.09	5.01	1.02	0.68
	0.26	4.65	4.55	1.02	1.20
	0.40	4.57	4.45	1.03	1.21
	0.53	4.38	4.26	1.03	1.31
	0.67	4.34	4.22	1.03	1.31
	0.80	4.23	4.12	1.03	1.46
	1.07	4.16	4.13	1.01	0.56
Mono-N-dealkyl-disopyramide	0.13	4.54	4.29	1.06	2.41
	0.26	4.01	3.96	1.01	0.73
	0.40	4.05	3.95	1.02	1.09
	0.53	3.86	3.65	1.06	2.19
	0.67	3.73	3.58	1.04	1.58
	0.80	3.58	3.44	1.04	1.96
	1.07	3.44	3.32	1.04	1.86

*μ_{A1} and μ_{A2}, apparent mobility for the first and second enantiomer, respectively; α, enantioselective factor; R, resolution.

The influence of CD concentration on resolution followed the same pattern observed for the enantioselective factor, indicating that the difference in apparent mobility is the major factor affecting resolution.

Influence of Buffer pH

Another important factor for the chiral separation of drugs is the buffer pH. Under acidic conditions (pH below 5) the EOF is low and the negatively charged CD does not migrate through the cathode.[7] Under these conditions, neutral compounds, and even basic compounds, may not migrate to the detector side. Increasing the pH increases the EOF as well as the migration of sulfated β-CD, allowing the separation and detection of neutral compounds. Basic compounds can be analyzed in their ionizable or non ionizable forms depending on the pH selected for analysis.

In the present study, the neutral compounds were analyzed under basic conditions (Table 1) to obtain high EOF and because sodium deoxycholate, another chiral additive added to the running buffer, is not soluble at pH lower than 5.0.[9] The results for praziquantel and its metabolite in Table 3 showed that an increase in pH results in an increase in the apparent mobility of the drugs up to pH 9. Further increments in pH resulted in a reduction of this parameter. This behavior was difficult to interpret because the buffer pH affects the EOF and the electrophoretic mobility of both sulfated β-CD and sodium deoxycholate. Better resolutions were obtained by increasing the pH.

The results obtained for the analysis of the basic compounds were very interesting because they showed that the enantioseparation can be obtained in the pH range of 4.0 to 10.0 depending on the drug. The maximum chiral separation for fluoxetine and norfluoxetine was obtained at pH 10.0, while mexiletine and their metabolites were better separated at pH 7.0. On the other hand, disopyramide and its metabolite could only be separated on pH lower than 7.0. Considering a pKa value of about 10.6 for these alkylamines,[33] we expect at least disopyramide, mexiletine, and their metabolites to be separated in their protonated form. The apparent mobility increased by increasing the running buffer pH for all drugs and metabolites studied as a result of an increase in the EOF. The enantioselective factor decreased with pH. As can be seen in Table 3, the resolution of mono-N-dealkyldisopyramide was severely affected by the running buffer pH.

Influence of Running Buffer Concentration

Increasing the buffer concentration will reduce the EOF due to a reduction in the zeta potential, resulting in increased migration times. However, when

Table 3. Influence of Running Buffer pH

Drug/Metabolite	pH	μ_{A1} ($\times 10^{-4}$)*	μ_{A2} ($\times 10^{-4}$)*	α*	R*
Praziquantel	8.0	2.22	2.14	1.04	0.65
	8.5	2.66	2.58	1.03	1.45
	9.0	2.80	2.71	1.03	1.51
	9.4	2.51	2.43	1.03	1.54
	10.0	2.53	2.47	1.02	1.51
Trans-4-hydroxy-praziquantel	8.0	2.89	2.76	1.05	1.38
	8.5	3.24	3.11	1.04	2.51
	9.0	3.38	3.22	1.05	2.39
	9.4	3.16	3.02	1.05	2.36
	10.0	3.19	3.07	1.04	2.24
Disopyramide	4.0	3.43	3.32	1.03	1.77
	4.5	3.82	3.70	1.03	1.59
	5.0	4.16	4.06	1.02	1.85
	5.5	4.71	4.56	1.03	1.75
	6.0	4.84	4.74	1.02	1.19
	7.0	5.65	5.55	1.02	1.26
Mono-N-dealkyl-disopyramide	4.0	2.92	2.79	1.05	2.00
	4.5	3.24	3.09	1.05	1.84
	5.0	3.51	3.35	1.05	1.62
	5.5	4.13	3.94	1.05	1.59
	6.0	4.03	3.91	1.03	1.43
	7.0	4.80	4.80	1.00	0

*μ_{A1} and μ_{A2}, apparent mobility for the first and second enantiomer, respectively; α, enantioselective factor; R, resolution.

resolving charged solutes using charged CD, the electrostatic interaction with the CD should also be considered. This charged selector also contributes to the current and, consequently, to the Joule effect. If excessive Joule heat is not removed by improper temperature control at high field strengths, the peak efficiency will decrease, thus reducing resolution. In addition, increasing the buffer concentration will modify the hydrophobic interaction with CD, also resulting in different resolution.[6,15]

In the present study, we observed a slight increase in the apparent mobility of mexiletine and its metabolites, as can be seen in Table 4. Resolution was also slightly affected by the buffer concentration. These experiments were carried out at pH 7.0 and at this pH the drug and metabolites are protonated, so that the electrostatic interaction with the negatively charged CD should also be considered. Increasing the buffer concentration decreased this interaction, resulting in a reduction in migration times.

Table 4. Influence of Running Buffer Concentration

Drug/Metabolite	Buffer Concentration (mmol/L)	μ_{A1} ($\times 10^{-4}$)*	μ_{A2} ($\times 10^{-4}$)	α	R
Mexiletine	10	1.90	1.81	1.05	1.18
	15	1.94	1.85	1.05	1.25
	20	2.09	1.99	1.05	1.28
Hidroxymethylmexiletine	10	2.39	2.35	1.02	0.73
	15	2.42	2.38	1.02	0.82
	20	2.60	2.56	1.02	0.81
p-Hydroxymexiletine	10	1.57	1.45	1.08	1.65
	15	1.61	1.49	1.08	1.74
	20	1.78	1.61	1.10	1.94

*μ_{A1} and μ_{A2}, apparent mobility for the first and second enantiomer, respectively; α, enantioselective factor; R, resolution.

Influence of Temperature and Voltage

Changes in the capillary temperature can also lead to different effects on chiral separation, because the temperature affects buffer viscosity and the formation of the complexes between the analyte enantiomers and the chiral selector.[6] In the present study, an increase in temperature has an increasing effect on the apparent mobility and a reducing effect on the enantioselective factor for fluoxetine and its metabolite (Table 5).

Table 5. Influence of Temperature and Voltage

Drug/Metabolite	Temp. (°C)	Voltage (KV)	μ_{A1} ($\times 10^{-4}$)*	μ_{A2} ($\times 10^{-4}$)	α	R
Fluoxetine	18		2.62	1.82	1.44	7.45
	20		2.83	1.99	1.42	7.07
	25		3.45	2.48	1.39	6.64
Nor-fluoxetine	18		3.09	2.12	1.46	11.39
	20		3.32	2.33	1.42	11.19
	25		3.99	2.89	1.38	10.18
Albendazole sulfoxide		15	2.36	2.33	1.01	1.10
		20	2.81	2.78	1.01	0.99
		25	3.57	3.54	1.01	0.88

*μ_{A1} and μ_{A2}, apparent mobility for the first and second enantiomer, respectively; α, enantioselective factor; R, resolution.

When the voltage was increased the apparent mobility of the drugs increased and the enantioselective factor did not change. The behavior observed for albendazole sulfoxide is shown in Table 5. The reduction in the resolution could be explained by the increase in Joule heat which reduces peak efficiency.[30]

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