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Chiral analysis of selected dopamine receptor antagonists in serum using capillary electrophoresis with cyclodextrin additives

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Abstract

Dopamine D-2 receptor antagonists' eticlopride and sulpiride were determined in serum using capillary electrophoresis with cyclodextrin additives. Chiral resolution of $S(-)$ and $R(+)$ sulpiride and eticlopride were achieved using 2% sulfated-β-cyclodextrin (S-β-CD) in 20 mM citrate run buffer (pH 2.90). A 72-cm fused silica capillary operated in the reversed polarity mode voltage of 20 kV was used for the analysis. The analytes of interest were isolated from serum using a solid phase extraction procedure with recoveries in excess of 85% for all four enantiomers. The D-2 receptor antagonist $(-)$ butaclamol was used as internal standard. The limits of detection were 0.3 and 0.1 μg/ml for $S(-)$ and $R(+)$ eticlopride and for $S(-)$ and $R(+)$ sulpiride, respectively, in 1 ml of serum. The limits of quantitation were 2 and 1 μg/ml for $S(-)$ and $R(+)$ eticlopride, and for $S(-)$ and $R(+)$ sulpiride, respectively. Calibration curves were linear over the 2–20 μg/ml range for eticlopride and 1–20 μg/ml range for sulpiride. The coefficients of determination were greater than 0.99 ($n = 12$ for eticlopride and $n = 15$ for sulpiride). Precision and accuracy of the method were 0.27–6.38 and 0.20–3.60% for $S(-)$ eticlopride, 2.33–4.28 and 0.80–5.73% for $R(+)$ eticlopride, 3.46–6.84 and 0.80–4.26%, for $S(-)$ sulpiride; and 4.71–6.47 and 2.00–6.67% for $R(+)$-sulpiride, respectively. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Dopamine D-2 receptor antagonist; Sulpiride; Eticlopride; Butaclamol; Capillary electrophoresis; Substituted cyclodextrins; Chiral separations

1. Introduction

Enantiomers of chiral drugs often show different pharmacological and toxicological properties; therefore determination of individual enantiomers is necessary for monitoring the pharmacokinetics and pharmacodynamics of chiral drugs. Usually, only one enantiomer is responsible for the biologic function of a given racemic compound. In some cases, both enantiomers exhibit activity whereas for some solutes a correct ratio of the enantiomers is necessary for optimal response [1].

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Because of recent regulations by the Food and Drug Administration concerning the enantiomeric purity of drugs and chemicals, enantioselective synthesis and the development of analytical methods to separate racemates are very important [2]. Capillary electrophoresis (CE) is considered to be a significant complementary technique to liquid chromatography (LC) and gas chromatography (GC) [3]. Advantages of CE are high efficiency, short analysis time, low consumption of chiral additives in the run buffer, and fast equilibration time upon changing the buffer composition. Low injection volumes can also be quite beneficial for bioanalytical applications especially in the in vivo monitoring of biofluids due to less stressing of the animal (or patient) with the potential of more samples taken per unit time. Small sample sizes are also beneficial in the case of combinatorial screening, which generates large number of samples in limited quantities.

A wide array of chiral selectors is presently available as additives to the run buffer in CE. Cyclodextrins (CDs) and derivatized CDs are among the most popular additives and are widely applicable, inexpensive and ultraviolet (UV) transparent. CDs are chiral, neutral oligosaccharides with a shape similar to a truncated cone with a relatively hydrophobic cavity able to form inclusion complexes with analytes. The outside surface of a CD is hydrophilic. The formation of inclusion complexes between enantiomers and CDs is strongly influenced not only by the hydrophobic interaction in the cavity, but also by the interaction between the hydroxyl groups (or other substituents) on the rim of CDs and substituents near the asymmetric center of the analyte.

A sulfated-β-cyclodextrin (S-β-CD) is negatively charged over the entire pH range and moves opposite to EOF in CE. Therefore, a reverse polarity is often applied for separations when using this type of chiral additive. Some chiral pharmaceuticals have been separated with S-β-CD with detection at the cathodic end of an untreated fused-silica capillary under neutral and basic conditions [4]. In the reverse polarity mode with decreased selector concentration, not only migration times but also selectivity and ionic strength of the buffer solution increased. Normally, the weaker the host–guest interaction, the faster the migration times. However, when detecting at the anode in the reverse polarity mode, the weaker the interaction between the chiral selector and the solutes, the longer the migration times. Previous studies of S-β-CD have shown that it is capable of separating large numbers of structurally diverse neutral and cationic analytes [5,6].

In recent years, there has been considerable interest in substituted benzamides as new antipsychotic agents. This interest is derived from clinical observations that medicines in this class caused fewer extrapyramidal side effects than do neuroleptics of the butyrophenone type [7]. There is evidence that suggests that many of the substituted benzamides are antagonists of dopamine receptors not linked to the adenylate cyclase system but to dopamine D-2 receptors. These receptors mediate the antipsychotic effect of the neuroleptics. A close correlation has been found between the dopamine D-2 receptor inhibitory potencies of neuroleptics measured by in vitro receptor binding techniques and their clinical potencies. Antipsychotic agents block the D-2 receptor stereoselectively, and their binding affinity is very strongly correlated with clinical antipsychotic and extrapyramidal potency.

Sulpiride and eticlopride are substituted benzamides. Sulpiride is also a neuroleptic agent and although it has some sedative effect in humans, it is not inhibitory at low dose [8]. It has been suggested that the lack of extrapyramidal side effects by sulpiride is due to a preferential effect on limbic than striatal tissue. The hydrophilic properties of sulpiride may also account for its limited penetration into the central nervous system and its low potency. Assays of sulpiride in plasma have been performed using HPLC, GC, TLC, radioimmunoassay and spectrometry [9–15]. A stereoselective HPLC assay for $S(-)$ and $R(+)$ sulpiride in serum has been reported [16]. The active enantiomer of sulpiride is the $S(-)$ enantiomer and it is a popular dopamine D-2 receptor antagonist. Its relatively low potency and poor ability to penetrate into

the brain has been a drawback in pharmacological and in vivo binding studies and has limited the usefulness of [^{3}H] sulpiride as a marker for central dopamine D-2 receptors [7].

Eticlopride is a new highly selective high-affinity dopamine D-2 receptor antagonist [7,17–19]. The active $S(-)$ eticlopride is closely related to $S(-)$ sulpiride and potentially modifies animal behavior, whether spontaneous or induced. Eticlopride has a potential clinical use in the management of psychotic states as an antipsychotic drug.

This paper reports the enantiomeric separations of $S(-)$ and $R(+)$ eticlopride and sulpiride at low μg/ml concentrations in serum using capillary electrophoresis with S-β-CD in the citrate run buffer. Solid phase extraction is utilized as a serum clean-up. There have been no previous reports of enantiomeric separation of these analytes by CE.

2. Material and methods

2.1. Instrumentation

The CE experiments were performed on an ABI Model 270A instrument (Applied Biosystems, Foster City, CA). Separations were performed on a 72 cm (50 cm to the detector) × 50 μm ID fused-silica capillary (Polymicro Technologies, Phoenix, AZ), applying a potential of 20 kV in the reverse polarity mode. The capillary was thermostated at 25°C with air coolant. The detection window was created by stripping the polyimide coating of the capillary. The detection wavelength for all analytes was 220 nm. New capillaries were conditioned with 0.1-N NaOH for 20 min followed by 10 min of deionized water. This was also performed before starting each day's run. Between runs, the capillary was rinsed with 0.1-N NaOH for 2 min, and run buffer for 3 min. The citrate buffer was prepared using 20 mM citric acid adjusted to the appropriate pH with 200 mM sodium citrate after the addition of appropriate amounts of S-β-CD. All the samples were hydrodynamically injected for 5 s into the CE instrument.

2.2. Chemicals and reagents

Sulfated-α-cyclodextrin (S-α-CD), S-β-CD, β-cyclodextrin (β-CD), hydroxypropyl-γ-cyclodextrin (HP-γ-CD), hydroxypropyl-α-cyclodextrin (HP-α-CD), heptakis (2,6-Di-*O*-Methyl)-β-cyclodextrin (DM-β-CD), and heptakis (2,3,6-tri-*O*-methyl)-β-cyclodextrin (TM-β-CD) were obtained from Aldrich Chemical Company (Milwaukee, WI) and were used as received. Taurocholic sodium, α-CD, and taurodeoxycholic sodium salt were obtained from Sigma Chemical Company (St. Louis, MO). Methyl-β-cyclodextrin (M-β-CD), HP-β-CD, γ-CD, carboxymethyl-β-cyclodextrin (CM-β-CD) were gifts from Cerestar (Hammond, IN, USA). Highly sulfated β-cyclodextrin (HS-β-CD), highly sulfated α-cyclodextrin (HS-α-CD) and highly sulfated γ-cyclodextrin (HS-γ-CD) were purchased from Beckman Coulter (Fullerton, CA). Tris buffer, ultragrade sodium citrate, and citric acid were obtained from Sigma Chemical Company. Concentrated phosphoric acid and sodium hydroxide were purchased from J.T.Baker (Phillipsburg, NJ). Sodium borate was purchased from Fisher Scientific (Fair Lawn, NJ). All chemicals and reagents were of the highest grade commercially available. Deionized water was purified using a cartridge system (Picotech Water System, RTP, NC). Drug free serum was obtained from Biological Specialty (Colmar, PA). Varian C18 and Nexus solid phase extraction cartridges (100 mg/ml) and the Vac-Elut vacuum manifold were obtained from Varian Sample Preparation Products (Harbor City, CA, USA). Oasis HLB cartridges were purchased from Waters Corp (Milford, MA). J&W C18 cartridges (100 mg/ml) were obtained from J&W Scientific (Folson, CA). All solutions were filtered through a 0.22-μm nylon filter (Alltech Associates, Deerfield, IL).

Except for S-CD concentrations of 2% w/v, all other CD and bile salts concentrations were 20 and 30 mM, respectively, in the run buffer. The pH of the run buffer was adjusted to 2.90 after dissolving the CD or appropriate derivative in the buffer. All run buffers were freshly prepared and filtered prior to analysis.

The (+) and (−) Butaclamol, S(−) and R(+) sulpiride and S(−) and R(+) eticlopride were purchased from RBI (Natick, MA, USA). The chemical structures of the analytes studied are shown in Fig. 1.

2.3. Preparation of stock and spiked solutions of analytes

Sample stock solutions of each enantiomer were prepared in absolute methanol to give concentrations of 1 mg/ml and were stored at −20°C. A solution of (−)-butaclamol (internal standard) was also prepared in absolute methanol–water (50:50 v/v) at a concentration of 0.5 mg/ml. To generate calibration curves, appropriate volumes of the individual analyte solutions were pipetted into 1.7 ml plastic centrifuge tubes (Dot Scientific Incorporated, Burton, MI), 20 µl internal standard solution added, followed by drug free serum to 1 ml and mixing by vortex mixer.

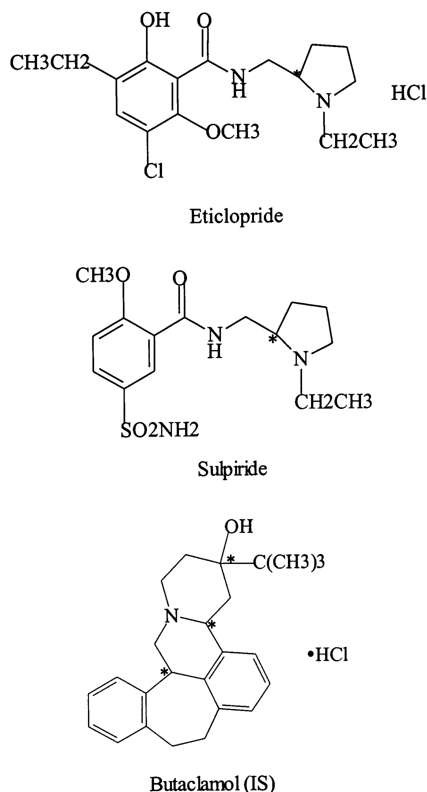

Fig. 1. The chemical structures of analytes studied.

2.4. Assay procedure

Serum sample clean-up was performed using solid phase extraction (SPE). Varian C18, J&W C18, Oasis HLB and Nexus HLB cartridges were investigated. The Oasis cartridge was washed with 1 ml water–methanol (95:5, v/v) after application of a serum sample. All other cartridges were washed with 2 × 1-ml deionized water. The cartridges were eluted with either 1 ml methanol containing 0.5% TFA or 1 ml acetonitrile containing 0.5% TFA. The Nexus HLB cartridges eluted with acetonitrile containing 0.5% TFA provided the highest recoveries of analytes (See Table 1). There were interfering peaks observed at the migration time of eticlopride using the Oasis cartridge, and this allowed the Nexus cartridge to be used in the SPE procedure.

The internal standard (−) butaclamol stock solution measuring 20 µl was added to 1 ml spiked serum samples containing R(+) and S(−) sulpiride and R(+) and S(−) eticlopride. The samples were mixed and passed through the Nexus SPE column attached to a vacuum manifold. The SPE column was washed with 2 × 1-ml of water and was allowed to dry in air for 3 min. The enantiomers and internal standard were then eluted with 1 ml of 0.5% v/v TFA in acetonitrile. Tubes were centrifuged under vacuum conditions and eluting solvent was evaporated. The samples were reconstituted in 1 ml of distilled water–methanol (8:2 v/v) after which each sample was filtered and hydrodynamically injected onto the capillary for 5 s.

Calibration curves were constructed using concentrations of 1, 2, 8, 10, and 20 µg/ml for S(−) and R(+) sulpiride and 2, 8, 10, and 20 µg/ml for S (−) and R(+) eticlopride. Linear regression analysis of drug/internal standard (D/IS) peak area ratios versus analyte concentrations gave slope and intercept data, which were used to calculate the concentration of each analyte in the serum samples. For absolute recovery experiments, spiked samples were compared to unextracted stock solutions of each analyte that was injected directly into the CE. D/IS peak area ratios were also used to calculate absolute recoveries of each analyte.

Table 1
Extraction efficiency of D-2 receptor antagonists in human serum using SPE

Analytes	Mean Recovery (%) [a]				
	$S(-)$ eticlopride	$R(+)$ eticlopride	$S(-)$ sulpiride	$R(+)$ sulpiride	$(-)$ Butaclamol
Nexus ACN-TFA[b]	91.9	94.7	87.0	87.2	85.0
MEOH-TFA[c]	89.1	87.8	65.3	63.5	95.7
C18 ACN-TFA[b]	80.5	79.2	68.2	66.1	96.8
Varian MEOH-TFA[c]	90.9	87.1	66.2	65.1	93.7
C18 ACN-TFA[b]	91.8	90.4	46.6	46.6	101.0
J&W MEOH-TFA[c]	81.5	79.3	65.0	68.0	85.6
Oasis[d] ACN-TFA[b]	85.2	82.7	69.9	73.5	100.9
MEOH-TFA[c]	95.9	94.4	93.2	88.7	100.3

[a] $n = 3$; 1 ml of serum contained 5 μg/ml of each analyte and 10 μg/ml of internal standard.
[b] Analytes are eluted using 1 ml acetonitrile containing 0.5% TFA.
[c] Analytes are eluted using 1 ml methanol containing 0.5% TFA.
[d] Oasis cartridge was washed with 1 ml 5% methanol–deionized water (v/v). All other cartridges were washed with 1 ml deionized water.

3. Results and discussion

3.1. Optimization of CDs and other chiral additives

Resolution of $S(-)$ and $R(+)$ eticlopride, $S(-)$ and $R(+)$ sulpiride and $(-)$ butaclamol internal standard was attempted using various neutral and charged CDs and bile salts. Neutral cyclodextrins studied were α-CD, β-CD and γ-CD and their derivatives, HP-γ-CD, HP-β-CD, HP-α-CD, DM-β-CD, TM-β-CD, M-β-CD. The charged CDs investigated were S-β-CD, S-α-CD, CM-β-CD, HS-β-CD, HS-α-CD and HS-γ-CD. The bile salts taurocholic sodium and taurodeoxycholic sodium were also studied. No separations were obtained using bile salts and CDs except for HS-β-CD and S-β-CD in 20 mM citrate buffer at pH 2.9 where baseline separation of all four enantiomers was achieved within 20 min. S-β-CD was chosen for the assay because of the high cost of the proprietary HS-β-CD compound.

The degree of substitution (d.s., 7–11% w/v) of S-β-CD was used to describe the concentration. Optimization of CD in the run buffer included the influence of CD type and concentration and d.s. Both resolution and migration times of the enantiomers were strongly influenced by the S-β-CD concentration. An adequate CE separation occurred in the midrange concentration. Higher affinity for one of the enantiomers for the CD is a result of better steric orientation in the CD analyte complex. This leads to a longer incorporation time of this stereoisomer in the CD cavity and to a decreased migration time with the influence of chiral selector in the reverse polarity mode. Similar to the normal polarity mode, the concentration of CD at maximum resolution is dependent on the chiral compound being separated and on the type of CD used. As the concentration of S-β-CD increased (see Table 2), the mean migration times shortened and the system current increased dramatically with decreased resolution of the analytes. When the concentration of S-β-CD was above 3% w/v, baseline drift became a serious problem. When the S-β-CD concentration was lower than 2%, migration time reproducibility was not good enough for quantitation. Thus 2% S-β-CD was selected as the best concentration of the chiral additive in the run buffer.

At pH 2.9, the polyanionic S-β-CD complexed with cationic analytes and effectively reversed the sign of average mobility to create a counter-EOF setup. The countercurrent migration of S-β-CD favored the enantioresolution of cationic type analytes. The application of charged CDs in CE

expands the resolution window in comparison with neutral CDs. They offer much higher flexibility in optimizing separation. They can be used with enhanced selectivity because of high hydrogen bonding capacity and also offer electrostatic interaction via ion pairing with analytes of opposite charges due to their charged mode. The migration times for the enantiomers tended to decrease as the concentration of S-β-CD increased. Most decreases in migration times were probably due to increased interactions between S-β-CD, the analytes and a decreased EOF. According to equilibrium theory, increased concentration of S-β-CD resulted in an increase in the amount of complexed analytes. Because of the polyanionic nature of S-β-CD, the complexed analytes have shorter migration times than free positively charged analytes in the reverse polarity mode. Also, the presence of S-β-CD can affect the EOF by altering the ionic strength as well as the conductivity and viscosity of the buffer. Increases in ionic strength and viscosity tend to decrease the EOF and migration times. The electrophoretic resolution of analytes decreased with increasing S-β-CD concentration, but the migration time was much shorter. Low resolution and increased current limited the benefit of continued increases in S-β-CD concentration. Since sulpiride and eticlopride contains two amine moieties at low pH, both amines will be protonated and they can interact electrostatically with the sulfate groups in S-β-CD.

3.2. Type of run buffers and pH

Since the pH of the run buffer in CE affects the selectivity and resolution of chiral separations, a careful investigation of analyte structure was nec-

Table 2
Effect of various parameters on the migration time and resolution of dopamine D-2 receptor antagonists using S-β-CD as chiral additive

Parameter	Rs	Eticlopiride migration time (min)		Rs	Sulpride migration time (min)	
		$S(-)$	$R(+)$		$S(-)$	$R(+)$
Chiral selector concentration (% w/v) (pH 2.90, 20 mM citrate)						
1	7.31	8.64	10.04	2.14	23.17	23.75
2	5.79	8.22	9.27	1.06	18.29	18.65
3	5.56	8.16	9.11	1.04	18.06	18.33
5	5.26	8.00	8.95	1.01	17.78	18.42
Buffer concentration (mM) (pH 2.90, 2% S-β-CD)						
10	4.85	7.94	8.87	0.86	17.20	17.50
20	5.79	8.22	9.27	1.06	18.29	18.65
30	6.38	8.58	9.86	1.21	23.01	23.63
50	7.53	9.34	10.92	1.39	28.74	29.50
75	10.9	10.27	12.45	1.65	41.60	43.07
pH (20 mM citrate, 2% S-β-CD)						
2.60	5.23	7.97	8.91	1.01	17.46	17.77
2.90	5.79	8.22	9.27	1.06	18.29	18.65
3.25	7.90	8.73	9.99	1.35	23.09	23.62
4.10	11.0	11.21	14.11	–[a]	–[a]	–[a]
Voltage (kV) (20 mM, pH 2.90 citrate, 2% S-β-CD)						
15	5.40	11.14	12.48	0.85	24.46	24.90
20	5.79	8.22	9.27	1.06	18.29	18.65
25	6.10	6.24	7.03	1.09	14.71	14.98
30	6.46	4.99	5.45	1.10	12.36	12.59

[a] No peaks for $S(-)$ and $R(+)$ sulpiride within 40 min.

essary at the beginning of assay development. The pH of the buffer had a pronounced effect on the separation. Intermediate pH values between pH 2.60 and 4.10 were investigated. The effect of run buffer pH on migration times and resolution of the enantiomers is shown in Table 2. Migration time and resolution decreased as buffer pH decreased. This was expected because ionizable analytes are more positively charged at low pH. The increased charge led to a stronger ionic interaction with anionic S-β-CD, which in turn resulted in a decrease of migration time and resolution. At higher pH, EOF is beginning to play a role, particular for the more weakly bound sulpiride. When the pH was higher than 3.25, baseline drift was again noted. At pH 2.60, the resolution between analytes and internal standard was not baseline. Thus, pH 2.90 was selected as the pH for the assay.

Various run buffers such as 20 mM citrate, 35 mM phosphate–borate, 100 mM Tris buffer and 50 mM phosphate buffer with 2% w/v S-β-CD were prepared at pH 2.5. There was no separation for sulpiride using Tris buffer. There were no sulpiride peaks within 60 min using the phosphate–borate buffer. Migration time was not reproducible and run time was too long using the phosphate buffer. Only citrate buffer gave baseline separation of the enantiomers within 20 min.

The concentration of buffer could have a pronounced effect on resolution. Increasing buffer strength will modify both the hydrophobic and electrostatic interactions to analyte/CD complex. It could occur by promoting the inclusion of a solute hydrophobic moiety into the interior cavity of the CD. In the reverse polarity mode, shorter migration times provided a shorter time for enantiomer chiral selector discrimination. Migration times and resolution for analytes increased as the concentration of citrate buffer increased (Table 2). When buffer concentration was higher than 30 mM, baseline drift became serious. Low buffer concentration caused buffer depletion effect, and gave irreproducible migration time. In this study, 20 mM citrate buffer was chosen since it gave the best run times and analyte resolution. Also, an increase in run buffer ionic strength decreased ionic interactions between S-β-CD and the charged analytes.

3.3. Optimization of experimental parameters

In chiral and achiral assays by CE, system suitability parameters include sensitivity, resolution and migration time. The resolution remained essentially constant at low sample concentrations, but decreased at high sample concentrations. There are optimum combinations of CD concentrations with moderate sample concentrations and injection volumes to give the best peak separations.

The efficiency of the separations increased at higher voltage up to a certain level with the limitation of system current. Increasing the applied voltage can shorten migration times based on results seen in Table 2. When the applied voltage was higher than 20 kV, baseline drift became serious. Thus the voltage chosen for the assay was 20 kV.

In regard to stability of the sample stock solution, a conversion of $R(+)$ to $S(-)$ sulpiride can occur when the sample is prepared in water and stored at room temperature for more than 24 h. If kept in the refrigerator at 4°C, no conversion was observed. Thus stock standard $R(+)$ and $S(-)$ sulpiride solutions were prepared in methanol and kept at −20°C. These solutions were stable for at least one month.

3.4. Quantitative analysis

To increase the limits of detection of the analytes, an on-capillary sample concentration technique was employed. All samples were prepared in deionized water–methanol (80:20 v/v), whose conductivity was lower than that of the run buffer. Upon application of 20 kV, a greater field developed across the sample zone causing the ions to migrate faster. When the ions reached the run buffer, the field decreased and they migrated slower. This process continued until the analytes were compressed into a small zone.

The quantitative aspects of the method were examined and the results are shown in Table 3. Percent error was higher in the 15 μg/ml samples for both analytes; but they showed better precision. Repeatability studies were performed with a 5 μg/ml concentration of each analyte at six injec-

Table 3
Accuracy and precision of serum samples spiked with $S(-)$ and $R(+)$ sulpiride and $S(-)$ and $R(+)$ eticlopride

	Concentration added (μg/ml)	Concentration found (μg/ml)	RSD (%)	Percent error (%)
Intra day[a]				
$S(-)$ eticlopride	5	5.04 ± 0.32	6.38	0.72
	15	14.69 ± 0.52	6.05	2.07
$R(+)$ eticlopride	5	5.06 ± 0.12	2.33	1.12
	15	14.14 ± 0.60	4.28	5.73
$S(-)$ sulpiride	5	5.06 ± 0.34	6.84	1.20
	15	14.60 ± 0.50	3.46	2.67
$R(+)$ sulpiride	5	5.16 ± 0.33	6.47	3.20
	15	14.22 ± 0.69	4.88	5.20
Inter day[b]				
$S(-)$ eticlopride	5	4.99 ± 0.30	6.08	0.20
	15	14.82 ± 0.48	3.27	3.60
$R(+)$ eticlopride	5	4.96 ± 0.17	3.38	0.80
	15	14.28 ± 0.53	3.74	5.47
$S(-)$ sulpiride	5	5.04 ± 0.31	6.09	0.80
	15	14.36 ± 0.53	3.68	4.26
$R(+)$ sulpiride	5	5.10 ± 0.31	6.14	2.00
	15	14.00 ± 6.60	4.71	6.67

[a] Based on $n = 6$.
[b] Based on $n = 9$.

tions. The RSD% for repeatability of migration time was less than 3% for both sulpiride and eticlopride. The limits of detection (LOD) for S (−) and R (+) sulpiride and $S(-)$ and $R(+)$ eticlopride were determined to be 0.1 and 0.3 μg/ml, respectively (S/N = 3). The limits of quantitation (LOQ) were 2 μg/ml for $S(-)$ and $R(+)$ eticlopride and 1 μg/ml for $S(-)$ and $R(+)$ sulpiride based on a S/N = 10. In order to achieve better sensitivity for both analytes, an analyst will have to use larger volumes of serum and employ a concentration steps for the SPE clean up. In this fashion, concentrations of 50–100 ng/ml can be achieved for quantitation.

Specificity was tested by adding each of the enantiomers into the blank serum. A typical electropherogram shown in Fig. 2 showed that the specificity of the system was excellent based on baseline separations of all the enantiomers.

Calibration curves for $S(-)$ and $R(+)$ sulpiride were prepared in a concentration range of 1–20 μg/ml and curves for $S(-)$ and $R(+)$ eticlopride were prepared in the range of 2–20 μg/ml using (−) butaclamol as internal standard. The coefficients of determination were greater than 0.99 ($n = 12$ for eticlopride and $n = 15$ for sulpiride) for each curve (See Table 4). The plot of peak areas versus concentration of analytes showed good linearity.

4. Conclusion

HS-β-CD and S-β-CD were found to be useful chiral additives for the CE separation of sulpiride and eticlopride enantiomers from serum in a single injection. The successful separations at low pH in the citrate run buffer indicated that the ionic interactions were between the protonated amine and the anionic sulfated-CD. S-β-CD was selected for the study based on cost.

S-β-CD was found to be most effective for enantiorecognition under acidic conditions, probably due to the relatively high degree of substitution of the sulfate group. Both ionic interaction and inclusion may contribute to the enantiorecognition.

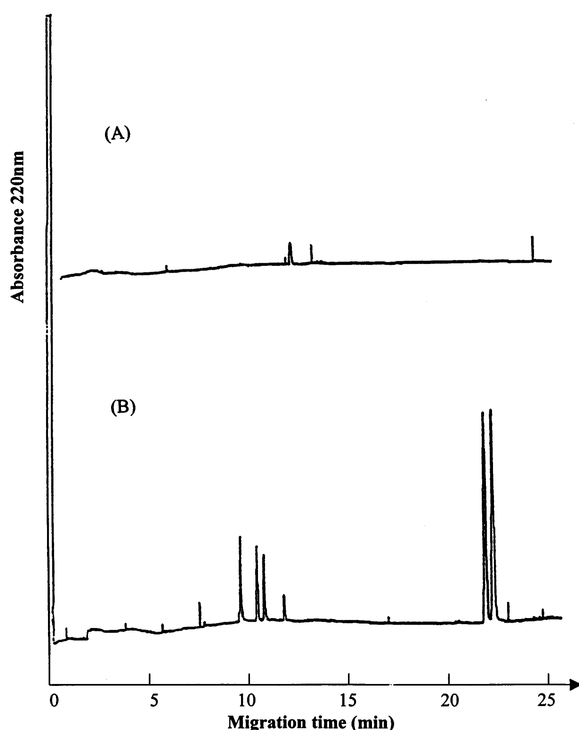

Fig. 2. Representative electropherograms of $S(-)$ and $R(+)$ sulpiride and $S(-)$ and $R(+)$ eticlopride using 2% w/v S-β-CD in citrate run buffer pH 2.90; (A) blank serum; (B) spiked samples at concentrations of 5 μg/ml.

Table 4
Coefficients of determination data for eticlopride and sulpiride in spiked serum

r^2	Eticlopride		Sulpiride	
	$S(-)$	$R(+)$	$S(-)$	$R(+)$
Day 1[a]				
Run 1	0.9977	0.9898	0.9987	0.9970
Run 2	0.9980	0.9979	0.9961	0.9922
Run 3	0.9985	0.9980	0.9970	0.9994
Day 2[b]	0.9930	0.9924	0.9912	0.9910
Day 3[b]	0.9900	0.9908	0.9922	0.9921

[a] Based on $n = 12$ for both curves of eticlopride and $n = 15$ for both curves of sulpiride.

[b] Based on $n = 12$ for each curve of eticlopride and $n = 15$ for each curve of sulpiride.

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