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Development of a capillary electrophoresis assay for the determination of carvedilol enantiomers in serum using cyclodextrins

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Abstract

A capillary electrophoresis method using cyclodextrins as the chiral selectors was developed for the determination of carvedilol enantiomers in serum. Several types of cyclodextrins were evaluated. The effect of cyclodextrin concentration on enantiomer resolution was investigated. Best results were obtained using 10 mM hydroxypropyl-β-cyclodextrin in the run buffer. The effect of voltage on efficiency was assessed. Other electrophoretic conditions were optimized. The method was validated for carvedilol enantiomers in serum. Linearity of detection was assessed over the concentration range of 50–4000 ng/ml of each enantiomer in serum. Intra- and inter-assay variability obtained were under 8% for both enantiomers.

Keywords: Carvedilol; Capillary electrophoresis; Analysis enantiomers stereoselective

1. Introduction

Carvedilol, 1-(4-carbazolyloxy)-3-[2-(2-methoxy)ethylamino]-2-propanol (Fig. 1), is a new antihypertensive drug recently introduced in the market. It is a non-selective β-adrenergic receptor antagonist and an α_1-adrenoceptor blocker [1–3]. The β_1-blockade produces a decrease in heart rate and in the force of contraction of the cardiac muscle. The α_1-antagonism results in relaxation of the smooth muscle in the blood vessels causing a decrease in peripheral vascular resistance [1,4]. At higher concentrations (>1 μM) carvedilol also exhibits calcium channel antagonist activity [2]. Although this effect occurs at doses higher than the typical clinical dose, it is possible that it contributes to the vasodilatory action of carvedilol, at least in cutaneous and renal vascular beds [5,6]. It has also been reported that carvedilol exhibits cardioprotective effects, with a marked reduction of infarct size observed in animal models of acute myocardial infarction [7].

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Carvedilol contains a single chiral centre in its structure and therefore exists as two enantiomers. The drug is administered as the racemate, however the two enantiomers possess different pharmacological actions. (+)-(S)-Carvedilol is a much more potent β_1-blocker than (+)-(R)-carvedilol, whereas both enantiomers exhibit the same α_1-adrenergic antagonism [8,9].

The methods reported in the literature for the analysis of the enantiomers of carvedilol consist of derivatization of the drug with optically pure derivatizing reagents to form diastereomers which are then analyzed by HPLC using either adsorption or partition mode with fluorometric detection. Optically pure derivatizing reagents such as (+)-(R)-phenylethyl isocyanate (PEI), 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) and (+)-(S)-naproxen chloride have been used [10–15]. Attempts to derivatize carvedilol with achiral fluorescent reagents, namely 2-naphthoyl chloride and 2-anthroyl chloride were undertaken in our laboratory. These reagents were used in early investigations in our laboratory for the analysis of mexiletine enantiomers and were shown to increase the limit of quantitation of the assay for that drug [16,17]. However, the reaction with carvedilol enantiomers produced incomplete reaction and multiple derivatization at both the secondary amino group on the side chain and at the hydroxyl group (confirmed by mass spectrometry). Therefore, the need for a simple and direct stereoselective assay that would not rely on derivatization methods was evident.

Capillary electrophoresis (CE) has become a very attractive alternative analytical tool for the determination of drug enantiomers. The high efficiencies obtained with CE provide enantiomeric resolution with shorter analysis times, compared with HPLC. Furthermore, CE offers the advantage of a variety of efficient chiral selectors at lower costs compared to chiral HPLC columns, as well as low consumption of solvents and reagents.

The most widely used chiral selectors in CE are the cyclodextrins. Cyclodextrins are cyclic oligosaccharides consisting of several glucose units connected to form a cyclic structure. They have the shape of a cone in which the internal diameter is determined by the number of glucose units present (usually 6, 7 and 8, corresponding to α, β and γ-cyclodextrin). The cavity is hydrophobic while the entrance to the cavity is lined with secondary hydroxyl groups and is thus hydrophilic [18]. Interaction with a solute will occur by inclusion of a hydrophobic portion of the solute in the cavity and from hydrogen bonding to the chiral hydroxyl groups of the rim [19–21].

The objective of this study was to determine the applicability of CE for the analysis of carvedilol enantiomers in serum. The use of native and substituted cyclodextrins as chiral selectors will be investigated and the electrophoretic conditions for the assay will be optimized.

2. Experimental

2.1. Materials and supplies

(±)-Carvedilol, (+)-(R)-carvedilol, and (-)-(S)-carvedilol were kindly donated by Boehringer Mannheim GmbH (Mannheim, Germany). (-)-Propranolol was obtained from Ayerst Laboratories (Montreal, PQ). All other chemicals and reagents were of analytical grade. Fused silica capillaries, uncoated, 57 cm length (50 cm effective length), 75 μm I.D. were purchased from Beckman (Palo Alto, CA). Hydroxypropyl-β-cyclodextrin, dimethyl-β-cyclodextrin, β-cyclodextrin, and γ-cyclodextrin were also obtained from Beckman (Palo Alto, CA). Heptakis ((2,3,6-tri-*O*-methyl)-β-cyclodextrin) was purchased from Sigma (St Louis, MO). Samples were filtered through 0.2 μm Acrodisc LC13 PVDF filters (Gelman, Ann Arbor, MI) prior to injection into the capillary electrophoresis apparatus.

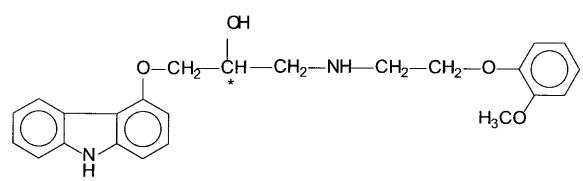

Fig. 1. Chemical structure of carvedilol. The asterisk denotes the chiral centre.

2.2. Equipment

Capillary electrophoresis was performed on a P/ACE System 5000 instrument (Beckman Instruments, Fullerton, CA) equipped with a UV detector with four filters. Data were collected and analyzed on a 486/66 Hz computer (Dell Computer Corporation, Austin, TX) equipped with System Gold Chromatography Software, version 8.10 (Beckman Instruments).

2.3. Electrophoretic conditions

The electrophoretic analyses were performed using a 75 μm I.D. fused silica capillary, 57 cm (50 cm to the detector) in length. The background electrolyte consisted of phosphate buffer, pH 2.5, and cyclodextrins as the chiral selectors. Samples were injected into the capillary by pressure, for 4 s. The electrophoresis was carried out by applying high voltage to the capillary, with the cathode being at the detector end. The capillary was washed between runs with a sequence of rinses: 0.1 M sodium hydroxide (1 min), water (0.5 min), 0.1 M hydrochloric acid (0.5 min), and water (0.5 min), to ensure reproducibility of the assay. Before sample injection, the capillary was rinsed with the run buffer for 1 min. Detection was by UV at 200 nm. Buffer ionic strength, pH, type and concentration of cyclodextrin, as well as applied voltage were optimized. The appropriate UV filter was selected and the optimum cartridge temperature determined. The migration order of the enantiomers was determined by injecting a solution of the racemate enriched with each enantiomer separately. The first peak to pass the detector window was determined to be (+)-(R)-carvedilol, followed by (−)-(S)-carvedilol. Corrected peak area (area/migration time) ratios of drug/internal standard were used for quantitations.

2.4. Choice of chiral selector

Several types of cyclodextrins (native and derivatized forms) in different concentrations, were tested in order to obtain separation of carvedilol enantiomers. A stock solution of racemic carvedilol (10 μg/ml) in ethanol was injected into the capillary. The cyclodextrins and concentrations examined were: γ-cyclodextrin, at 10 and 50 mM, β-cyclodextrin, at 3 and 15 mM, dimethyl-β-cyclodextrin, at 5 and 20 mM, trimethyl-β-cyclodextrin, at 10 mM, and hydroxypropyl-β-cyclodextrin, at 10 and 50 mM.

2.5. Effect of cyclodextrin concentration on resolution

Resolution of the enantiomers was compared using various concentrations of cyclodextrin in the run buffer. Phosphate buffers containing 5, 10, 20, and 50 mM of hydroxypropyl-β-cyclodextrin were prepared, and capillary electrophoresis of racemic carvedilol in ethanol (10 μg/ml) was performed. Resolution factors (R_s) for the enantiomers were calculated and the optimal cyclodextrin concentration for the analysis determined.

2.6. Effect of voltage on efficiency

The effect of the applied voltage on the efficiency of separation of carvedilol enantiomers by capillary electrophoresis was examined over the 10–22 kV range. A solution of racemic carvedilol in ethanol (10 μg/ml) was used for the injections. Efficiency of the assay, expressed as the number of theoretical plates (N) for the peak corresponding to (R)-(+)-carvedilol (first enantiomer detected), was calculated using the formula $N = 16\ (l/w)^2$, where $N =$ number of theoretical plates, $l =$ migration distance and $w =$ peak width. A plot of N versus voltage was constructed and the optimum voltage identified.

2.7. Effect of buffer ionic strength on efficiency

The effect of buffer concentration on the separation efficiency of carvedilol enantiomers was assessed by performing the electrophoretic separation using phosphate buffer, pH 2.5, at 10, 15, 20, 25, and 50 mM, with 10 mM hydroxypropyl-β-cyclodextrin as the chiral selector.

2.8. Effect of cartridge temperature on resolution

The temperature inside the capillary cartridge may affect separation in capillary electrophoresis because it affects solute mobility and the electroosmotic flow (EOF) [22]. At the operating pH of 2.5 EOF is minimal. Therefore temperature will solely affect electrophoretic mobility. In order to determine the optimum temperature for the resolution of carvedilol enantiomers, electrophoretic runs at different cartridge temperatures (15, 18, 20 and 25°C) were performed, and the separation of the enantiomers examined.

2.9. Extraction procedure

Blood was collected in additive-free Vacutainer tubes, allowed to clot for 2 h at room temperature, and then submitted to centrifugation at 3000 rpm for 20 min. Serum was stored at −20°C until required for analysis. Prior to the experiments, serum was adjusted to pH 7.4 with sodium phosphate salts (5.2 mg sodium phosphate monobasic monohydrate and 43.4 mg sodium phosphate dibasic heptahydrate in 1 ml of serum). Racemic carvedilol was added to serum to obtain the desired concentration and an aliquot (200 μl) of internal standard solution (10 μg/ml of (-)-propranolol in HPLC-grade ethanol) was added. The samples (0.7 ml) were submitted to precipitation of proteins with 0.4 ml 10% trichloroacetic acid, basified with 0.4 ml of 1 M sodium hydroxide and subsequently extracted with 2 × 3 ml ethyl ether and vortex-mixing for 2 min. The extracts were evaporated to dryness under nitrogen. The residues were reconstituted in 200 μl HPLC-grade ethanol and filtered through 0.2 μm Acrodisc LC13 PVDF filters (Gelman, Ann Arbor, MI) for injection into the capillary.

2.10. Determination of detector linearity for carvedilol enantiomers in serum

Detector linearity for the CE assay was assessed in the serum concentration range of 50–4000 ng/ml per enantiomer. Samples were prepared in duplicate. Racemic carvedilol was added to serum (pH 7.4) to obtain final concentrations of 50, 100, 250, 500, 1000, 2000, and 4000 ng/ml of each enantiomer. The internal standard was added and samples were extracted and analyzed as described in the previous section. A calibration curve was constructed by plotting corrected peak area ratios of carvedilol enantiomers/internal standard versus the corresponding enantiomer concentration.

2.11. Determination of the reproducibility of the CE assay

Calibration curves consisting of concentrations of 50, 100, 500, 1000, and 2000 ng/ml of each enantiomer in serum were prepared in duplicate. Five replicate samples of carvedilol in serum at concentrations of 100 and 1000 ng/ml were also prepared. Samples (0.7 ml) were extracted and analyzed by the optimized CE method as described previously. Graphs of corrected peak area ratios of enantiomer/internal standard versus enantiomer concentration in serum were constructed and used to calculate carvedilol concentration in the samples. Intra-assay variability was expressed in terms of coefficient of variation (C.V.) of the determination, in percent, for the five replicates at each drug concentration. Inter-assay reproducibility was assessed by performing the same analysis described above on three different days.

3. Results and discussion

3.1. Choice of the chiral selector

Several types of cyclodextrins were initially tested in high and low concentrations in the run buffer, and resolution of carvedilol enantiomers was examined. γ-Cyclodextrin at 10 and 50 mM did not produce resolution of the enantiomers. This could be due to the large size of the cavity of the cyclodextrin (γ-cyclodextrin has eight glucose units) which did not allow stereoselective interaction with carvedilol. Partial resolution was obtained with 3 and 15 mM β-cyclodextrin, as can be observed in Fig. 2. This cyclodextrin has a smaller cavity, as it contains only seven glucose units in its structure, apparently providing better

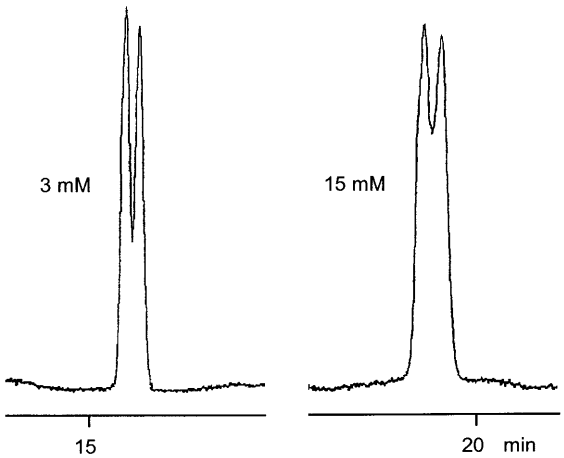

Fig. 2. Separation of racemic carvedilol at 10 μg/ml in ethanol using 3 and 15 mM β-cyclodextrin as the chiral selector in the run buffer. Electrophoretic conditions: buffer: 25 mM sodium phosphate, pH 2.6; capillary: fused silica, uncoated, 57 (50) cm length, 75 μm I.D.; voltage: 20 kV; temperature: 20°C; injection: pressure, 5 s; detection: UV at 214 nm.

interaction of the enantiomers of carvedilol. Resolution of carvedilol enantiomers has also been recently reported by Koppenhoefer et al. [23] using a polyacrylamide coated capillary and a run buffer consisting of 100 mM sodium phosphate, pH 2.5, and 15 mM β-cyclodextrin.

Substitution at the secondary hydroxyl rim on the surface of the cyclodextrin can dramatically affect selectivity of the separation, as it will provide additional interaction points with the analyte [19,22,24]. A substituted β-cyclodextrin, dimethyl-β-cyclodextrin, was tested, and the electropherograms of the separation obtained are shown in Fig. 3. When used at a concentration of 5 mM in the buffer, it produced partial resolution of the enantiomers. An increase in the concentration to 20 mM resulted in even less separation of the enantiomers. Soini et al. [25] reported resolution of carvedilol enantiomers using 10 mM dimethyl-β-cyclodextrin in a more complex run buffer (18 mM Trizma base, pH 2.9, 0.1% methylhydroxyethylcellulose 4000, 0.03 mM hexadecyltrimethylammonium bromide). Trimethyl-β-cyclodextrin, at 10 mM in the buffer, did not favor stereoselective interaction and a single peak was obtained for racemic carvedilol. Optimal results were obtained using a β-cyclodextrin substituted with hydroxypropyl groups. Hydroxypropyl-β-cyclodextrin, at 10 mM in the run buffer, produced very good separation of the enantiomers, although only partial resolution was obtained at 50 mM.

It has been postulated that the substitution of the secondary hydroxyl groups on the cyclodextrin rim with hydroxypropyl groups provides a less restricted hydroxyl group and at an appropriate length for hydrophilic interactions with the hydroxyl group at the chiral centre and the amine group close to the chiral centre for propranolol [26]. The authors observed improved resolution for propranolol enantiomers when changing the chiral selector from unsubstituted β-cyclodextrin to hydroxypropyl-β-cyclodextrin. The same explanation could be valid for carvedilol where the same effect of the type of cyclodextrin on resolution was obtained and in which the positions of the interacting groups are the same.

The substitution of the secondary hydroxyl groups on the rim with methyl groups as in the dimethyl-β-cyclodextrin also provides less re-

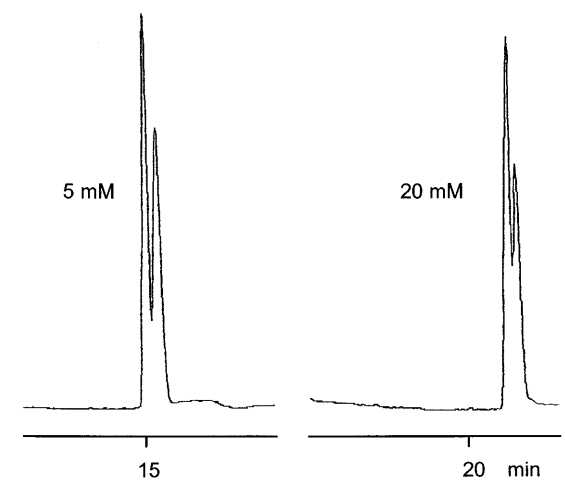

Fig. 3. Separation of racemic carvedilol at 10 μg/ml in ethanol using 5 and 20 mM dimethyl-β-cyclodextrin as the chiral selector in the run buffer. Electrophoretic conditions: buffer: 25 mM sodium phosphate, pH 2.6; capillary: fused silica, uncoated, 47 (40) cm length, 75 μm I.D.; voltage: 12 kV; temperature: 20°C; injection: pressure, 4 s; detection: UV at 200 nm.

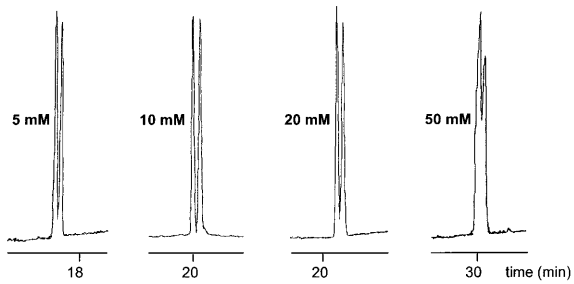

Fig. 4. Electropherograms of the separation of racemic carvedilol at 10 μg/ml in ethanol by CE using different concentrations of hydroxypropyl-β-cyclodextrin in the run buffer. Electrophoretic conditions: background electrolyte: 25 mM sodium phosphate buffer, pH 2.5; capillary: fused silica, uncoated, 57 (50) cm length, 75 μm I.D.; voltage: 14 kV; temperature: 20°C; injection: pressure, 2 s; detection: UV at 214 nm.

stricted interaction sites as compared to the unsubstituted native β-cyclodextrin. However, these interactions are hydrophobic, weaker interactions as compared to the interactions that can occur with the hydroxypropyl group, and therefore result in less enantioselectivity compared to that provided by hydroxypropyl-β-cyclodextrin [26].

3.2. Effect of cyclodextrin concentration on resolution

The optimum concentration of hydroxypropyl-β-cyclodextrin (HP-β-CD) for the separation of carvedilol enantiomers was determined. Fig. 4 shows the electropherograms corresponding to each HP-β-CD concentration tested, illustrating the effect of the chiral selector concentration on the resolution of carvedilol enantiomers.

As the HP-β-CD concentration increased from 5 to 10 mM, an increase in the separation of carvedilol enantiomers was observed. Maximum separation of the peaks was obtained at 10 mM HP-β-CD in the run buffer. Buffers containing ± 1 mM of the HP-β-CD concentration of maximum resolution (10 mM) were also tested and a slight decrease in resolution resulted. The existence of an optimum concentration of chiral selector, which enhances the difference between the apparent electrophoretic mobilities of the enantiomers, has been demonstrated by Wren and co-workers [27–29]. However, at cyclodextrin concentrations higher than the optimum, enantioselective interactions appear to be overwhelmed by non specific hydrophobic interactions and enantiomer resolution is precluded [30]. The migration time increased with an increase in cyclodextrin concentration. This is due to longer residence time of the drug in the complex form as well as to an increase in the viscosity of the buffer with a reduction of the mobility of the analytes. This has also been observed by other investigators for propranolol [28,31] and clenbuterol [32]. Table 1 presents the resolution factors (R_s) calculated for each electropherogram. Near baseline resolution, corresponding to an $R_s = 1.46$, was obtained using 10 mM HP-β-CD.

3.3. Effect of voltage on efficiency

In capillary electrophoresis, voltage plays a major role in determining analyses times. It can also affect the efficiency of analysis since efficiency is directly proportional to the voltage [33].

In order to determine the optimum separation voltage for the analysis of carvedilol by CE, several runs were performed with gradual increases in the applied voltage. Fig. 5 shows a plot of the calculated number of theoretical plates (N) versus voltage applied for the (+)-(R)-carvedilol peak. There was an increase in efficiency following an increase in applied voltage from 10 to 18 kV, with a maximum of 102 156 theoretical plates obtained at 18 kV. Further increases in voltage resulted in decreased efficiencies. The explanation for this is that the capillary becomes less effective in heat dissipation after a certain voltage level, where excessive Joule heat is generated [34]. As heat is

Table 1
Effect of hydroxypropyl-β-cyclodextrin concentration on the resolution of carvedilol enantiomers

Concentration (mM)	R_s
5	1.20
10	1.46
20	1.18
50	0.68

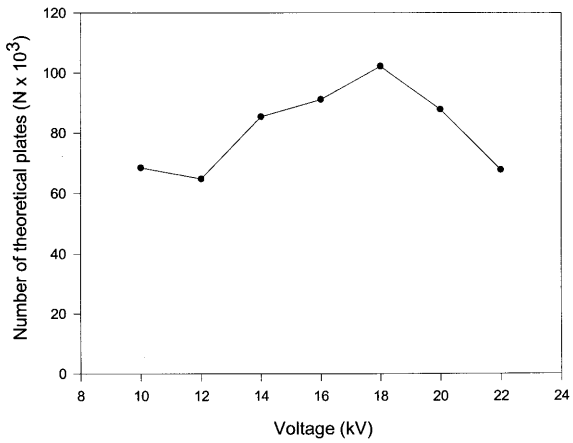

Fig. 5. Effect of applied voltage on efficiency of separation in the analysis of carvedilol by capillary electrophoresis. Electrophoretic conditions: background electrolyte: 25 mM sodium phosphate buffer, pH 2.5, 10 mM hydroxypropyl-β-cyclodextrin; capillary: fused silica, uncoated, 57 (50) cm length, 75 μm I.D.; temperature: 20°C; injection: pressure, 4 s; detection: UV at 200 nm.

produced inside the capillary, the viscosity of the buffer decreases, and sample diffusion resulting in peak broadening becomes significant. An applied voltage of 18 kV was thus selected for further analyses.

3.4. Effect of buffer ionic strength on efficiency

A series of buffer concentrations (10, 15, 20, 25, 50 mM phosphate, pH 2.5) were evaluated for the effect on efficiency of enantiomer resolution and migration times. Buffer concentrations from 10 to 25 mM resulted in similar resolution for carvedilol enantiomers with a gradual decrease in migration times. Efficiency and peak areas increased as the buffer ionic strength increased. This could be explained by a reduction in drug-wall interactions which can cause peak broadening and lower mass recovery. However, the use of buffer concentrations higher than 25 mM decreased greatly enantiomer resolution probably due to high current generated inside the 75 μm I.D. capillary. The use of high buffer concentrations would thus require the use of a capillary with smaller internal diameter. Based on these observations an optimum buffer concentration of 25 mM was selected for further analyses.

3.5. Effect of cartridge temperature on resolution

Capillary temperature control is extremely important for reproducibility of the assay. When current passes along a capillary, part of the electrical energy is converted into Joule heating. Temperature changes viscosity of the buffer and therefore the migration velocity of the analytes affecting the migration times and consequently the resolution of the analytes [32]. To control or minimize the effects of Joule heating, temperature can be controlled with fan-blown air or by a recirculating liquid, with the capillary mounted in a cartridge. The Beckman P/ACE 5000 equipment used for this study uses a circulating coolant containing perfluoro compounds, C_{5-18}, to maintain the temperature inside the capillary cartridge.

The resolution of carvedilol enantiomers decreased slightly with an increase in temperature from 15 to 25°C. Temperature may also influence the kinetics of the inclusion complex with the cyclodextrins. For example, the stability constant of the cyclodextrin inclusion complex would decrease with temperature, thus resulting in a decrease in resolution [24].

Little change in migration times was observed over the range of temperatures tested (15–25°C). However, at temperatures lower than 20°C, it was noticed that the capillary electrophoresis instrument was not as efficient in controlling the temperature, and that equilibration time was rather long. A convenient operational temperature of 20°C was thus selected for the analyses.

3.6. Optimized CE assay for carvedilol enantiomers in serum

The optimized electrophoretic conditions selected were: background electrolyte: 25 mM sodium phosphate buffer, pH 2.5, 10 mM hydroxypropyl-β-cyclodextrin; capillary: fused silica, uncoated, 57 (50) cm length, 75 μm I.D.; voltage: 18 kV; temperature: 20°C; injection: pressure, 4 s; detection: UV at 200 nm; internal standard: (−)-propranolol.

Ideally, in CE, the sample should be dissolved in an aqueous buffer to avoid differences in conductivity along the capillary length. However, the

serum extract containing carvedilol was not soluble in aqueous buffer, so it was necessary to reconstitute the extracts in ethanol for injection into the capillary. The sample plug of ethanol formed inside the capillary was poorly conductive, therefore the length of the sample zone (and consequently the injection time) was limited. It was observed that resolution of carvedilol enantiomers in ethanol decreased as the injection time increased. On the other hand, as one of the aims of analytical method development for carvedilol is high sensitivity, larger volumes of sample introduced into the capillary were desirable. Therefore, a balance between sample load, resolution and efficiency had to be achieved. An injection by pressure for 4 s was found to provide a reasonable sample load and maintain resolution.

Low operational pH was found to be essential for the resolution of carvedilol enantiomers. The stereoselective interaction with the cyclodextrins is highly dependent on the ionization state of the analyte. It has been shown by Vigh et al. [35,36] that three types of interactions can occur, depending on the ionization state of the analyte. In desionoselective separation (Type I), only the nondissociated form of the analyte interacts stereoselectively with the chiral selector. In the ionoselective type (Type II), only the dissociated form will produce enantioselective interaction. Duoselective separation (Type III) occurs when both dissociated and nondissociated forms of the enantiomers can combine in a stereoselective manner with the cyclodextrin [18,35–37]. In the case of carvedilol, which exhibits an ionoselective type of interaction, only the ionized form interacts stereoselectively with hydroxypropyl-β-cyclodextrin, therefore it was necessary to use a buffer with low pH to ensure that the drug was ionized.

Fig. 6 displays a representative electropherogram of an extracted sample of racemic carvedilol at 2 μg/ml in serum. Carvedilol enantiomers passed the detector window at 14.1 and 14.3 min, corresponding to (+)-(R)- and (−)-(S)-carvedilol, respectively. The internal standard migrated past the detector at 12.5 min. A separation time of 16 min was sufficient for the analysis which, when combined with the rinsing time (2.5 + 1 min), resulted in a total analysis time of 19.5 min. An injection of an extract from blank serum did not show any interfering peaks. In fact, components from serum were found to elute after 20 min of separation under 18 kV, which is later than the migration times of carvedilol and the internal standard. In CE, as soon as the substance of interest passes the detector window and is recorded as a peak, the separation can be stopped. The capillary can then be rinsed with appropriate solutions to remove all the remaining components of the sample thereby contributing to the faster analysis times observed in CE. The absence of major interference from the sample matrix is one of the advantages of CE. Direct injection of serum and saliva after an ultrafiltration procedure [38] and injection of urine samples after a simple filtration [39] have been reported using micellar electrokinetic capillary chromatography.

3.7. Determination of detector linearity for carvedilol enantiomers in serum

Excellent correlation was obtained between corrected peak area ratios and carvedilol concentra-

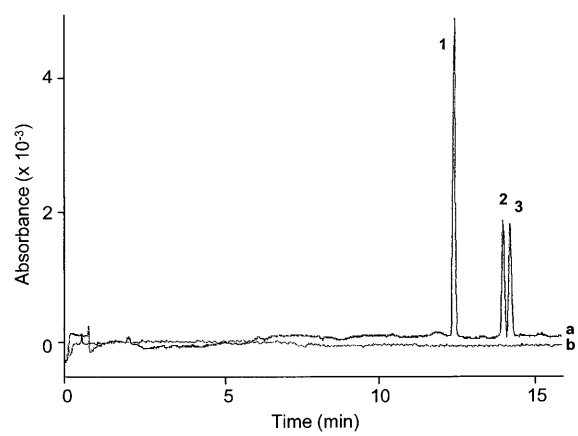

Fig. 6. Representative electropherogram of an extraction from serum. (a) Serum spiked with (±)-carvedilol (2 μg/ml) and internal standard; (b) blank serum; (1): (-)-propranolol, (2): (R)-(+)-carvedilol; (3): (S)-(-)-carvedilol. Electrophoretic conditions: background electrolyte: 25 mM sodium phosphate buffer, pH 2.5, 10 mM hydroxypropyl-β-cyclodextrin; capillary: fused silica, uncoated, 57 (50) cm length, 75 μm I.D.; voltage: 18 kV; temperature: 20°C; injection: pressure, 4 s; detection: UV at 200 nm.

Table 2
Concentrations obtained and intra- and inter-assay variability data for the determination of carvedilol enantiomers in serum by capillary electrophoresis

	100 ng/ml			1000 ng/ml		
	(R)-(+)	(S)-(−)	R/S	(R)-(+)	(S)-(−)	R/S
Day 1[a]	96.2	98.5	0.98	1016.2	983.7	1.03
SD	4.4	7.8		48.0	35.8	
C.V. (%)	4.6	7.9		4.7	3.6	
Day 2[a]	101.6	102.9	0.99	1019.7	997.3	1.02
SD	7.3	7.6		43.7	44.7	
C.V. (%)	7.2	7.4		4.3	4.5	
Day 3[a]	99.3	101.7	0.98	1004.4	978.7	1.03
SD	7.9	5.4		35.7	31.6	
C.V. (%)	8.0	5.3		3.6	3.2	
Average[b]	99.0	101.0	0.98	1013.4	986.6	1.03
SD	2.7	2.3		8.1	9.6	
C.V. (%)	2.7	2.3		0.8	1.0	

[a] Average of five replicates.
[b] Average of 3 days.

tion in the 50–4000 ng/ml per enantiomer range, with r^2 values of 0.999 for both enantiomers. The parameters of the lines obtained were: (+)-(R)-carvedilol: slope $= 4.06 \times 10^{-4}$, intercept $= -0.010$; (−)-(S)-carvedilol: slope $= 4.13 \times 10^{-4}$, intercept $= -0.014$. The limit of quantitation (LOQ) of the assay was 50 ng/ml per enantiomer in serum with a signal-to-noise ratio of 4. The stereoselective HPLC methods described in the literature report an LOQ of 1–2 ng/ml per enantiomer. Although the LOQ obtained by CE is higher it should be considered that the amounts of sample injected in CE are much smaller compared to HPLC. The volume of sample injected by pressure for 4 s was calculated to be approximately 18 nl. In HPLC volumes in the range of 1–100 μl are typically injected. Therefore the sensitivity limitations of the UV detection using CE is in fact due to the very small amounts injected.

3.8. Determination of the reproducibility of the CE assay

The intra-assay variability of the CE assay for the determination of carvedilol enantiomers in serum was determined by analyzing five replicate samples of carvedilol in serum at concentrations of 100 and 1000 ng/ml per enantiomer. Intra-assay variabilities were less than 8% at the lower concentration, and less than 5% at the higher concentration. Inter-assay reproducibility was determined by performing the analysis described above on three different days. Coefficients of variation were less than 3% at 100 ng/ml, and less than 1% at 1000 ng/ml per enantiomer in serum. The results obtained for intra- and inter-assay reproducibility are listed in Table 2.

4. Conclusions

In conclusion, an efficient stereoselective CE method was developed for the determination of carvedilol enantiomers in serum. The method uses a simple sodium phosphate buffer and hydroxypropyl-β-cyclodextrin as the chiral selector added to the run buffer. The assay produced baseline separation of the enantiomers with sharp peaks in a short analysis time. Method development was fast and inexpensive since only minor quantities of the chiral selectors were required for the preliminary tests. This study showed that CE can be a very valuable alternative to HPLC using chiral stationary phases or chiral derivatizing

reagents. Although limits of quantitation of the assay are still high it is reasonable to predict that the method could be used for stereoselective pharmacokinetics studies with the use of sample concentration techniques or more sensitive detector systems such as mass spectrometry or laser-induced fluorescence.

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