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Chiral separation of labetalol stereoisomers in human plasma by capillary electrophoresis

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Abstract

A newly derivatized cyclodextrin [octakis-(2,3-diacetyl-6-sulfato)-γ-cyclodextrin] was investigated as a chiral selector in capillary zone electrophoresis in a study of the chiral separation of labetalol stereoisomers. Heptakis(2,3-diacetyl-6-sulfato)-β-cyclodextrin (HDAS-β-CD) and octakis(2,3-diacetyl-6-sulfato)-γ-cyclodextrin (ODAS-γ-CD) were shown to be effective in separating labetalol stereoisomers. Optimal separating conditions of the four stereoisomers of labetalol were achieved with 10 mM HDAS-β-CD and 10 mM ODAS-γ-CD in an acidic pH buffer of low molarity. Data illustrating the effects of capillary length and cyclodextrin concentration on the separation are presented. The longer capillary length and high voltage enabled the baseline separation of all isomers in less than 15 min. The optimized method was applied to the analysis of human control plasma containing labetalol utilizing solid-phase extraction (SPE) in the 96-well format.

Keywords: Enantiomer separation; Labetalol; Cyclodextrins

1. Introduction

The importance of chirality in the pharmaceutical industry has long been recognized. Capillary electrophoresis (CE) has become popular and has proven to be a very useful technique due to the flexibility, high separation, efficiency, and enantioselectivity [1,2]. However, chiral separation of compounds with multiple stereogenic centers is more difficult than of the single chiral center compounds because chiral selector(s) must have the ability to differentiate several chiral centers simultaneously. The chiral selector must be soluble and chemically stable in the chosen run buffer, not exhibit a significant detector response, and form stereoselectively noncovalent diasteromeric complexes with enantiomers.

Labetalol was the first antihypertensive drug with both α- and β-adrenoreceptor blocking properties [3]. The compound possess two chiral centers and can exist in four stereoisomeric forms (*R*,*R*), (*S*,*S*), (*R*,*S*) and (*S*,*R*). The (*R*,*R*) isomer exhibits mainly a β_1-antagonist activity with some α_1-antagonism and β_2-agonist activity [4]. The (*R*,*S*) isomer appears to be pharmacologically inactive. In 1990, a single isomer (*R*,*R*) was marketed but was subsequently withdrawn due to hepatotoxicity [5]. Labetalol is currently marketed as a racemic mixture of all four stereoisomers. Hence, it is important to develop separation methods for chiral purity control of the drug and to study potential stereoselective differences in the metabolic disposition of labetalol stereoisomers and/or metabolites.

Extensive research has been conducted on the chiral separation of labetalol using different chiral selectors in both aqueous and nonaqueous media [6–10]. However, little work has been devoted to the complete chiral separation of all four stereoisomers of labetalol (Fig. 1). Aumatell et al. [11] have separated labetalol stereoisomers using 120 mM hydroxypropyl-β-cyclodextrin (HP-β-CD) in an acidic buffer. A complete separation of the four stereoisomers was demonstrated by Le Potier et al. [12] using 7.7 g/l sulfated-β-CD in a 30 mM phosphate buffer, pH 6.5. Vigh and co-workers used single isomer sulfated cyclodextrins to achieve full separation of labetalol stereoisomers with heptakis-(2,3-dimethyl-6-sulfato)-β-CD in a low-pH background electrolyte [8] and heptakis-(2,3-diacetyl-6-sulfato)-β-CD in a high pH background elec-

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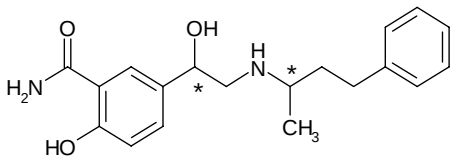

Fig. 1. Chemical structure of labetalol. Symbol (∗) denotes chiral centers.

trolyte [6]. Nonaqueous CE was also used by Vigh's group to separate labetalol stereoisomers in less than 40 min with acidic methanol running buffer [13]. Wang and Khaledi [14] utilized dynamically coated capillaries to improve efficiency and reduce the electroosmotic flow for enhancing resolution. However, only three of the four isomers of labetalol were separated with sulfated-β-CD. Tamisier-Karolak et al. [15] developed a separation for labetalol stereoisomers using capillary electrokinetic chromatography. A complete separation of the four isomers was obtained either at the cathode or anode depending on the pH of the background electrolyte. Few studies have been employed on the separation of labetalol isomers utilizing single isomers of β-cyclodextrin and γ-cyclodextrin as chiral additives in the CE running buffer [6,8,13,16]. Furthermore, no data have been reported on the capillary electrophoretic separation of labetalol stereoisomers extracted from human control plasma via solid-phase extraction (SPE).

The aim of this work was to investigate and compare the chiral selectors heptakis(2,3-diacetryl-6-sulfato)-β-cyclodextrin (HDAS-β-CD) and octakis(2,3-diacetryl-6-sulfato)-γ-cyclodextrin (ODAS-γ-CD) present in the running buffer on the chiral separation of labetalol stereoisomers by CE in a more reasonable timeframe. The effect of the cyclodextrin concentration added to the electrolyte, effective capillary length, and selectivity were examined. The optimized method was applied to human control plasma containing labetalol that was extracted using SPE in the 96-well format. Comparison of different SPE bonded phases was also investigated and the results are reported.

Pure analytical standards for all four stereoisomers of labetalol were not available. Therefore, the assignment of chirality for particular peaks obtained after chiral separation of all four stereoisomers and their migration order was not attempted.

2. Experimental

2.1. Apparatus

All capillary electrophoresis separations were carried out in 50 μm i.d. bare fused silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with length to the detector 49.5 cm and total length 56.4 cm (unless otherwise noted) that were installed in a P/ACE 5510 instrument (Beckman Instruments, Fullerton, CA, USA) equipped with a photodiode array detector. The detector was operated at 228 nm with a 10 nm bandwidth. The capillary temperature was thermostated at 25 °C. Samples were injected into the capillary by pressure (0.5 p.s.i. for 10 s; 1 p.s.i. = 6894.26 Pa). Electropherograms were acquired and processed by P/ACE Station software version 1.2 (Beckman Instruments, Fullerton, CA, USA).

2.2. Reagents

Phosphoric acid, lithium hydroxide, sodium hydroxide, and labetalol were purchased from Sigma–Aldrich (St. Louis, MO, USA). Acetonitrile was purchased from Emsco Scientific Enterprises (Philadelphia, PA, USA). HDAS-β-CD was purchased from Regis Technologies (Morton Grove, IL, USA). ODAS-γ-CD was purchased from Antek Instruments (Houston, TX, USA). The drug free heparinized human plasma was obtained from Biological Specialty Corp. (Lansdale, PA, USA). Buffers and standard solutions were prepared with Milli-Q water (Millipore, Bedford, MA, USA) and were filtered through a 0.2 μm pore size membrane filter (Gelman Laboratory, Ann Arbor, MI, USA).

2.3. Procedures

The background electrolyte was made by titrating 25 mM phosphoric acid whose pH was adjusted to 2.5 with aqueous lithium hydroxide. Using these buffer stock solutions 5, 10, 20, and 30 mM HDAS-β-CD and ODAS-γ-CD were prepared. Stock solutions of labetalol stereoisomers were prepared at a concentration of 100 μg/ml in acetonitrile (ACN)–water (50:50, v/v). New uncoated capillaries were conditioned with water for 30 min, followed by 0.1 M sodium hydroxide for 10 min, followed by water for 10 min, and then rinsed with running buffer for 10 min. Capillaries were preconditioned with running buffer for 1 min before injection and flushed with water for 1 min after each analysis.

The apparent electrophoretic mobility μ_{app} ($cm^2/V\,s$) of the solute was measured from the electropherogram using Eqs. (1) and (2):

$$\mu_{app} = \mu_{eff} + \mu_{EO} \quad (1)$$

$$\mu_{app} = \frac{L_d L_t}{V}\left(\frac{t_M - t_{ramp}}{2}\right) \quad (2)$$

where μ_{app} is the apparent mobility of the solute, L_d the effective length, L_t the total capillary length, V the applied voltage, t_M the migration time of the solute, and t_{ramp} is the voltage ramp time. The peak resolution (R_s) values were calculated from equation $R_s = 2\,(t_S - t_R)/(w_S + w_R)$, where t_S and t_R are the observed migration times and w_S and w_R are the observed peak widths of the stereoisomers on the baseline.

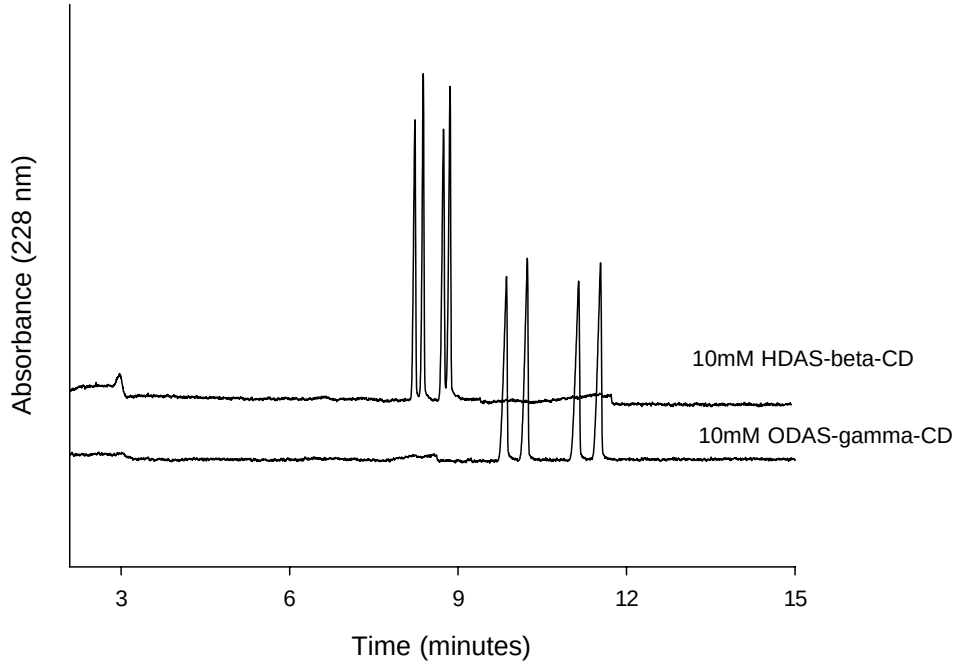

Fig. 2. Comparison of the separation of labetalol stereoisomers using CE with different chiral additives (voltage: 30 kV; capillary length: 56.4 cm; effective length: 49.5 cm).

2.4. Plasma extraction

A 500 μl aliquot of human control plasma lot 44556 was pipetted into a 13 mm × 75 mm polypropylene test tube, followed by addition of 50 μl labetalol standard (100 μg/ml) or 50 μl CAN–water (1:1) for the double blank. The samples were briefly vortexed, then a 500 μl aliquot of 0.15 M sodium phosphate (pH 2) was added to each tube, followed by an additional vortex mix. A 1 ml aliquot of the resulting solution was transferred to the 96-well method development SPE plate (ANSYS Technologies, Lake Forest, CA, USA) consisting of C_{18}AR, C_{18}, Phenyl, C_8, Cyano, C_2, MP1, and MP3, which had been conditioned with 500 μl methanol, followed by 500 μl of water and then 500 μl of 0.1 M sodium phosphate (pH 2). The sample solution was drawn through the plate by vacuum. The method development plate was subsequently washed with 1 ml of water, followed by 500 μl of 1 M acetic acid. The plate was then removed from the vacuum manifold and the bottom was rinsed with methanol to prevent cross-contamination of samples. The plate was centrifuged for 5 min at 1000 rpm (161 × g) to remove any residual liquid from the plate. The extraction plate was then placed onto a 0.65 ml deep-well 96-well collection plate and 300 μl of CH_2Cl_2–isopropanol–NH_4OH (78:20:2, v/v) was added to each well. The assembly was centrifuged for 5 min at 1000 rpm (161 × g) to elute the analyte into the collection plate. The eluates were evaporated to dryness under a stream of nitrogen at 40 °C. The residue was reconstituted in 200 μl of ACN–water (1:1). The wells in the collection plate were sealed using a thermal sealing foil. The plate was vortexed for 1 min, sonicated for 15 min, then vortexed for an additional 1 min. The collection plate was centrifuged for 5 min at 3000 rpm (1449 × g) to settle particulates. Finally, 150 μl of the supernatant was transferred to sample vials for CE analysis.

3. Results and Discussion

3.1. Comparison of HDAS-β-CD and ODAS-γ-CD

Previously, separation of all four labetalol stereoisomers was achieved using single-isomer sulfated CDs [6–8,13]. Labetalol stereoisomers were separated using 50 mM HDAS-β-CD in approximately 45 min [6]. In our work, separation of the four isomers of labetalol was achieved using 10 mM HDAS-β-CD or 10 mM ODAS-γ-CD in less than 12 min. Fig. 2 illustrates the comparison on the migration times of the stereoisomers. The size of the CD is an important factor in chiral recognition. The use of different size CDs ($\alpha > \beta > \gamma$) resulted in very different enantioselectivities [17]. Hence, the bigger cavity of ODAS-γ-CD resulted in better separation of the four stereoisomers of labetalol.

3.2. Effect of CD concentration

One of the most important parameters to consider in chiral CE analyses is the concentration of the chiral selector in the running buffer. The optimum concentration depends on the binding affinity of all the stereoisomers with the chiral selector. At a low-CD concentration, no separation of the stereoisomers is possible because there is not enough chiral

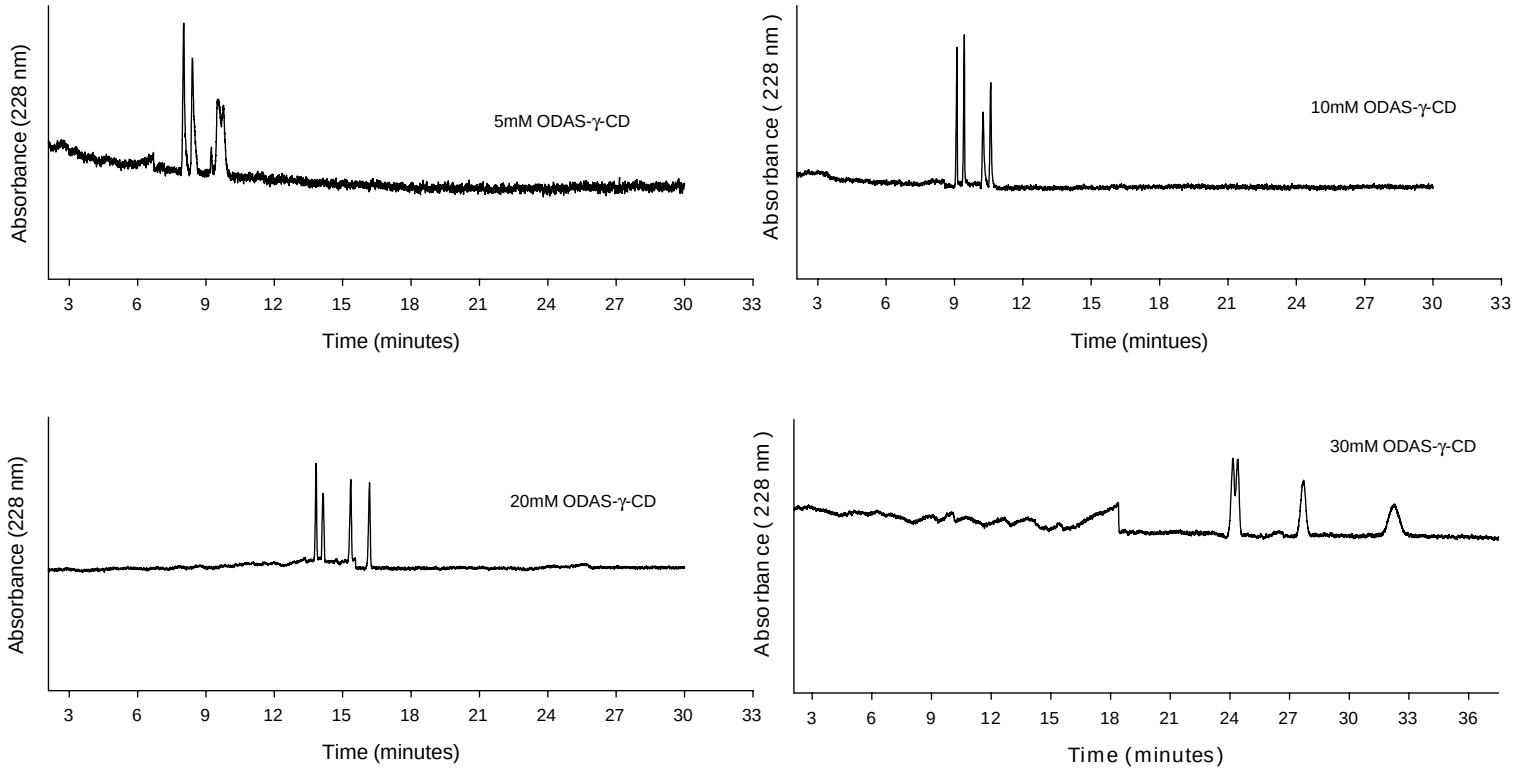

Fig. 3. Effect of varying the ODAS-γ-CD concentration on the chiral separation of labetalol stereoisomers (voltage: 30 kV; capillary length: 56.4 cm; effective length: 49.5 cm).

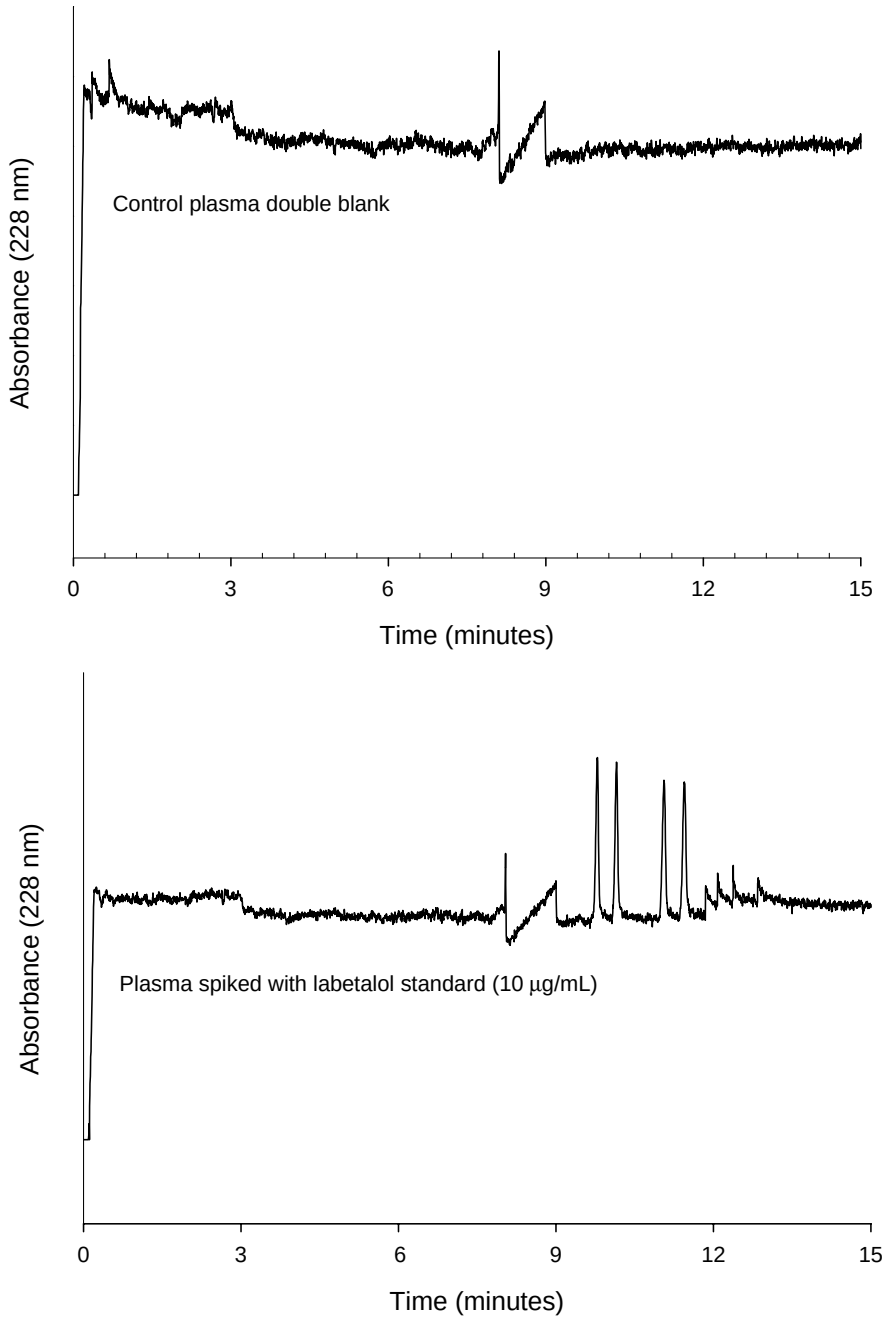

Fig. 4. Plasma extracts of labetalol stereoisomers utilizing SPE plate with C_{18}AR membrane analyzed in 10 mM ODAS-γ-CD (voltage: 30 kV; capillary length: 56.4 cm; effective length: 49.5 cm).

selector available to form the complexes. Conversely, at an extreme high concentration, all stereoisomers may be completely complex and no more separation may be observed [17]. Therefore, the optimum CD concentration should be determined to give the best separation for all stereoisomers studied.

Few studies have been reported on the chiral separation of labetalol stereoisomers using ODAS-γ-CD. This study was primarily focused on ODAS-γ-CD because it exhibits the greatest selectivity for labetalol and it has only recently become commercially available. Fig. 3 illustrates the effect on the separation of labetalol stereoisomers as the concentration of ODAS-γ-CD was varied from 5 to 30 mM.

3.3. Effect of capillary length

Using 10 mM ODAS-γ-CD for the separation of labetalol stereoisomers, the capillary length was varied to lengths of 30, 40, and 50 cm. The electrophoretic mobility of labetalol stereoisomers decreased with increasing capillary length because of the longer migration time to the detection window.

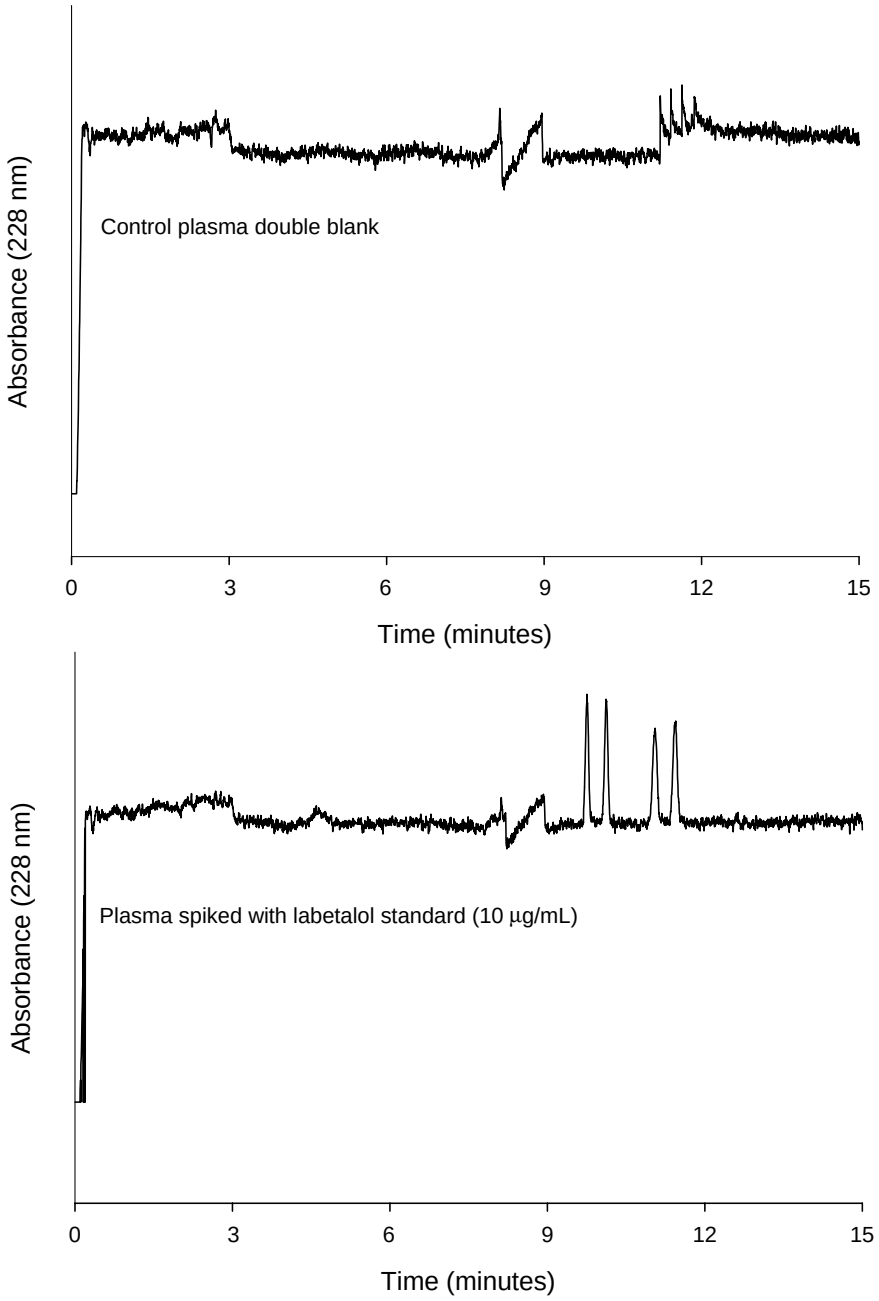

Fig. 5. Plasma extracts of labetalol stereoisomers utilizing SPE plate with C_{18} membrane analyzed in 10 mM ODAS-γ-CD (voltage: 30 kV; capillary length: 56.4 cm; effective length: 49.5 cm).

However, complete separation was achieved with a 50 cm capillary in less than 12 min.

3.4. Comparison of SPE phases

The SPE plate is manufactured with ultra-clean polypropylene plastic and bonded-silica impregnated on a glass fiber disc. Its low-bed mass, raw material purity, and optimized selectivity result in extracted samples which are free of contaminating peaks and matrix interferences. The 96-well SPE plate also permitted the rapid, parallel extraction of up to 96 samples. The method development plate consisted of C_{18}AR, C_{18}, phenyl, C_8, cyano, C_2, MP1, and MP3 phases. The C_{18}AR, C_{18}, phenyl, and C_8 membrane phases provided the cleanest sample extracts. Figs. 4–7 show the comparisons of plasma extracts obtained with these four bonded phases. The cyano, C_2, MP1, and MP3 membrane phases yielded extraction recovery that was qualitatively lower relative to the other membrane phases.

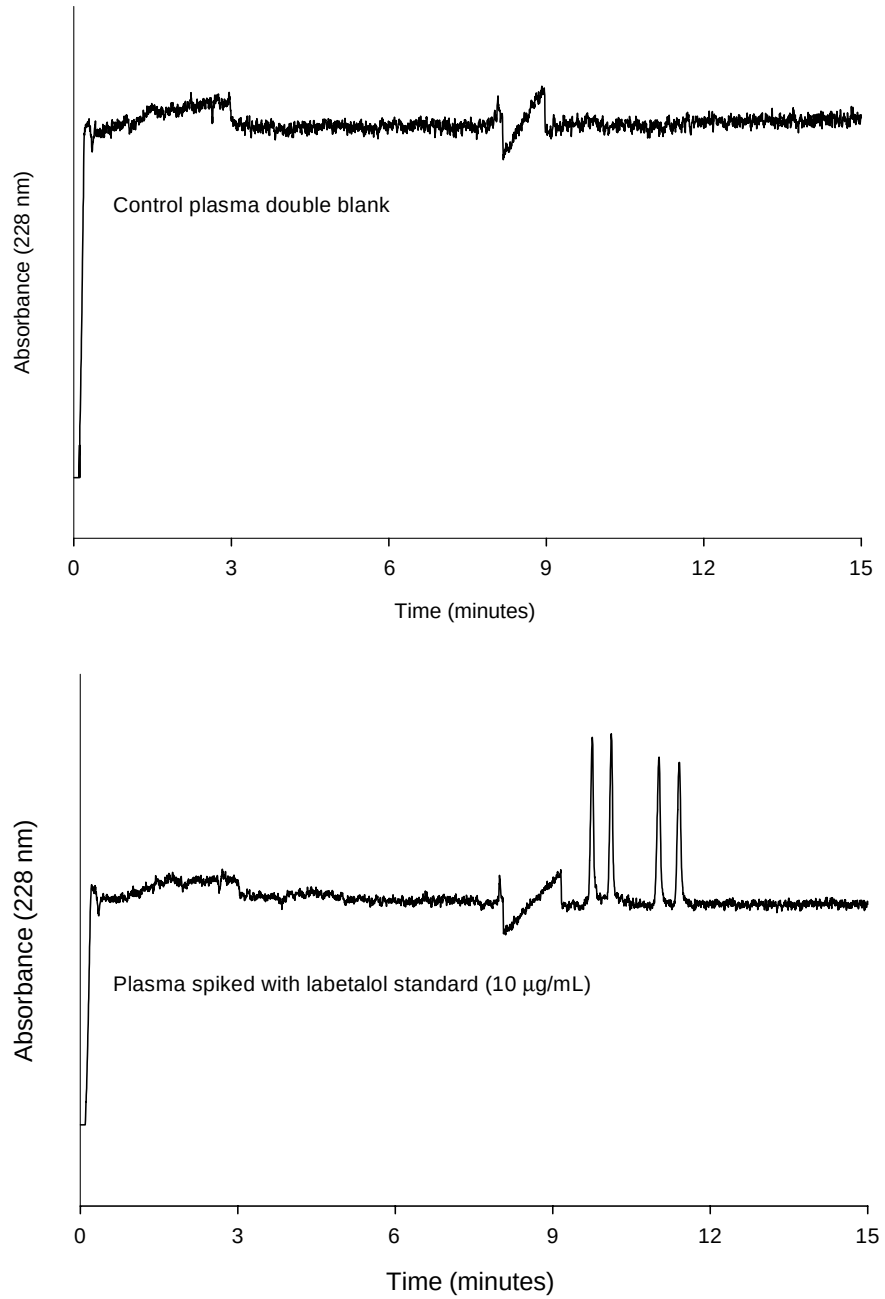

Fig. 6. Plasma extracts of labetalol stereoisomers utilizing SPE plate with phenyl membrane analyzed in 10 mM ODAS-γ-CD (voltage: 30 kV; capillary length: 56.4 cm; effective length: 49.5 cm).

The primary objective of this work was to describe an improved separation of labetalol stereoisomers using a newly commercialized single-isomer γ-cyclodextrin. The data presented here shows excellent selectivity for all four stereoisomers of labetalol in a shorter amount of time than has been previously achieved with other chiral additives [11–15]. The inclusion of the electropherograms of spiked plasma extracts illustrates the ability of the present method to yield good resolution for all four stereoisomers, free from endogenous interferences in actual biological samples similar to that obtained from pure reference standard solutions. The goal of this work was not intended to show quantitative data since rigorous validation was not performed. Further studies would need to be evaluated to determine important parameters such as the extraction efficiency, linearity, and plasma lot-to-lot variability in order to demonstrate the applicability of this method for the quantitative determination of labetalol stereoisomers in biological samples.

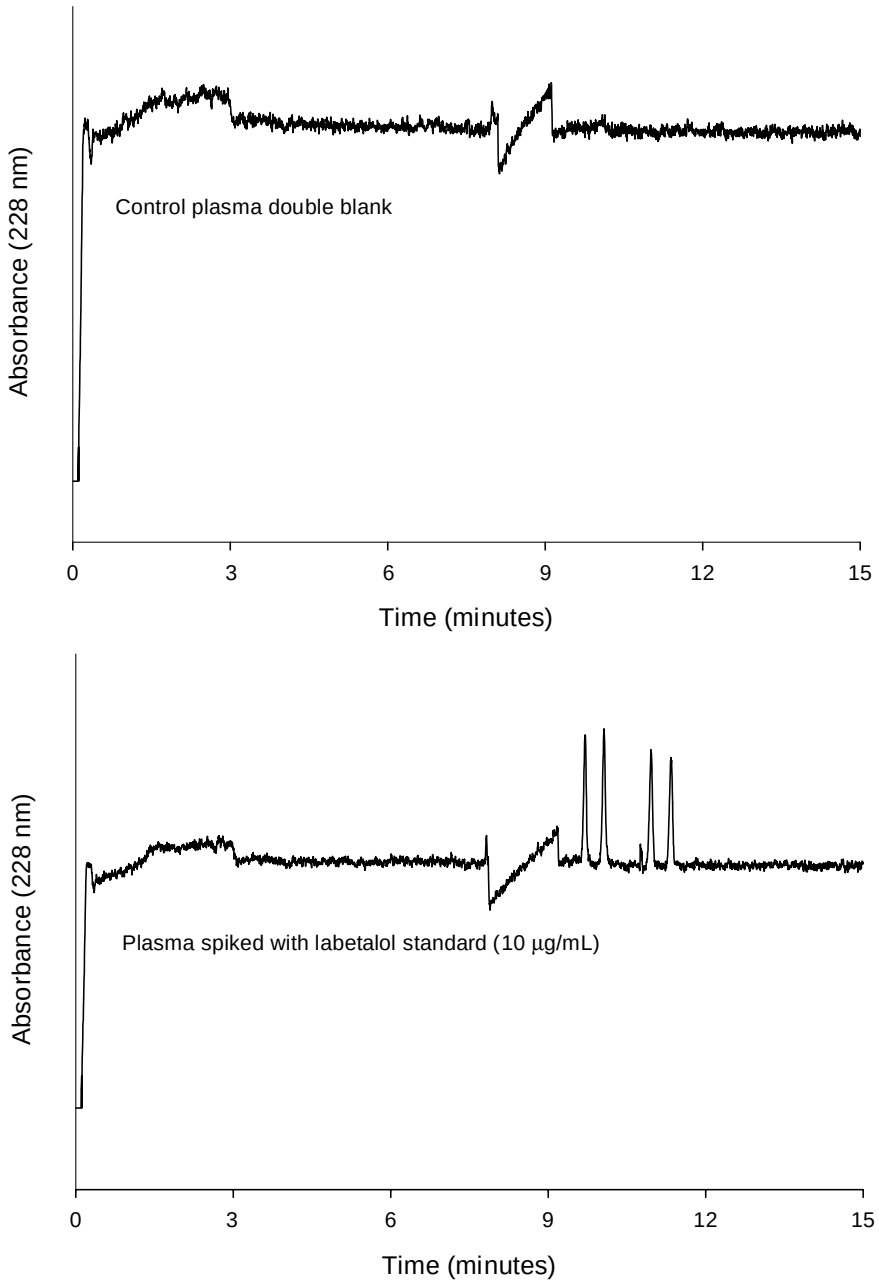

Fig. 7. Plasma extracts of labetalol stereoisomers utilizing SPE plate with C_8 membrane analyzed in 10 mM ODAS-γ-CD (voltage: 30 kV; capillary length: 56.4 cm; effective length: 49.5 cm).

4. Conclusion

It has been demonstrated that using a single-isomer octakis(2,3-diacetyl-6-sulfato)-γ-cyclodextrin in the CE running buffer a complete separation of all four stereoisomers of labetalol was achieved. ODAS-γ-CD afforded fast separations, with good peak resolutions. The described method was applied to labetalol-spiked control plasma following a 96-well sample preparation. The resulting extracts were free of any endogenous interferences, and complete separation of all four isomers was achieved in the extracted plasma samples.

References

[1] S. Fanali, J. Chromatogr. A 735 (1997) 77.
[2] H. Nishi, S. Terabe, J. Chromatogr. A 694 (1995) 245.
[3] J.B. Farmer, I. Kennedy, G.P. Levy, R.J. Marshall, Br. J. Pharmacol. 45 (1972) 660.
[4] E.H. Gold, W. Chang, M. Cohen, T. Baum, S. Ehrreich, G. Johnson, N. Prioli, E.J. Sybertz, J. Med. Chem. 25 (1982) 1363.
[5] V.A. Maria, R.M. Victorino, Ann. Pharmacother. 26 (1992) 924.
[6] J.B. Vincent, A.D. Solokowski, T.V. Nguyen, G. Vigh, Anal. Chem. 69 (1997) 4226.
[7] J.B. Vincent, D. M Kirby, T.V. Nguyen, G. Vigh, Anal. Chem. 69 (1997) 4419.
[8] H. Cai, T.V. Nguyen, G. Vigh, Anal. Chem. 70 (1998) 580.

[9] R. Gotti, V. Gavrini, V. Andrisano, G. Mascellani, J Chromatogr. A 845 (1999) 247.
[10] L. Valtcheva, J. Mohammad, G. Pettersson, S. Hjerten, J. Chromatogr. 638 (1993) 263.
[11] A. Aumatell, R.J. Wells, D.K.Y. Wong, J. Chromatogr. A 686 (1994) 293.
[12] I.L. Potier, S.L. Tamisier-Karolak, Ph. Morin, F. Megel, M. Taverna, J. Chromatogr. A 829 (1998) 341.
[13] W. Zhu, G. Vigh, J. Chromatogr. A 892 (2000) 499.
[14] F. Wang, M.G. Khaledi, J. Microcol. Sep. 11 (1999) 11.
[15] S.L. Tamisier-Karolak, M. Stenger, A. Bommart, Electrophoresis 20 (1999) 2656.
[16] M. Tacker, P. Glukhovskiy, H. Cai, G. Vigh, Electrophoresis 20 (1999) 2794.
[17] F. Bressolle, M. Audran, T.-N. Pham, J.-J. Vallon, J. Chromatogr. B 687 (1996) 303.