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Separation of Clenbuterol Enantiomers by Cyclodextrin-modified Capillary Electrophoresis(การแยกสารอิแนนทีโอเมอร์ของเคลนบิวเตอรอล โดยวิธี แคปิลารี...

Surapol Natakankitkul

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Separation of Clenbuterol Enantiomers by Cyclodextrin-modified Capillary Electrophoresis

Surapol Natakankitkul

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Chiangmai University, Thailand

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ABSTRACT: Cyclodextrin-modified capillary electrophoresis procedure for the enantiomeric separation of clenbuterol using hydroxypropyl-β-cyclodextrin as chiral selector was investigated. The enantiomeric drug investigated could be resolved with quick analysis and high resolution depending on many parameters such as chiral selectors, concentration of the chiral selector added, applied field strength, pH and buffer additives.

KEY WORDS: clenbuterol enantiomers, cyclodextrin-modified capillary electrophoresis

INTRODUCTION

The separation of enantiomers is widely studied in analytical chemistry especially in pharmaceutical and biological fields, since chiral drugs are administered either as enantiomers or as racemic mixtures (1). Thalidomide is an example which its enantiomers of the same recemic drug possess different pharmacological effects (2). While controversy at the Olympics about clenbuterol raises questions as to whether this sympathomimetic bronchodilator can affect muscle mass and function (3). Because of side-effects that could be caused by the presence of an undesirable component in a racemic drug, the current tendency in the pharmaceutical industry is to prepare drugs with one enantiomer only. However, the production of such drugs through stereoselective reaction or preparative enantiomeric separation can provide impure material. Thus, rapid sensitive analytical methods of high resolving power are required to control the synthetic chiral process of the pharmaceuticals. Principally, separation of optical isomers can be performed by two different strategies called direct and indirect chiral separation (4). Direct chiral separation involves the formation of diastereomeric molecule complexes of the two enantiomers with a chiral selector. For indirect enantio-separation both enantiomers undergo a derivatization reaction with a chiral selector to form diastereomers with distinct physico-chemical behavior. After the derivatization step, the diastereomers can be separated by using non-chiral chromatographic or electrophoretic systems. Analytical methods which have been used for the separation of chiral compounds include thin-layer chromatography (TLC) (5), gas chromatography (GC) (6), high performance liquid chromatography (HPLC) (7), and capillary electrophoresis (CE) (8-10). Recently, CE is a technique undergoing rapid development for chiral separation. A large number of chiral separations has been reported using CE or micellar electrokinetic chromatography (MEKC) (11-14). Furthermore, chiral separation via inclusion complexation by using

cyclodextrins (CDs) or their derivatives or crown ethers has been demonstrated (15-19). In addition, non-chiral reagents also play a significant role in improving chiral separations, including organic solvent, urea, bile salts and cationic surfactants and methyl cellulose (20-22). Rawjee et al. recently demonstrated a rational approach to predicting the electrophoretic mobility differences for enantiomers of weak acids and bases in CE using different natural and derivatized cyclodextrins as chiral selectors (23). The CE method for the separation of the enantiomers of basic drugs have also been published (23-27). In this work, the operating conditins were modified with hydroxypropyl-β-cyclodextrin (HP-β-CD) in a borated/phosphate electrolyte allowed the separation of clenbuterol enantiomers with high resolution and short run-time.

EXPERIMENTAL

Apparatus for CE

The analytical equipment consisted of a Quanta^{TM} 4000 (Waters Chromatography, Division of Millipore, Milford, MA, USA) capillary electrophoresis system connected with a system interface module and a personal computer. Data acquisition and processing was carried out with a commercial chromatographic software (Maxima 820). Uncoated, narrow-bore silica capillaries (AccuSep^{TM}) with an inner diameter of 50 μm, a total length of 32 cm, and an effective separation length of 24.5 cm each were used. On-column detection was performed at 214 nm.

Chemicals

Hydroxypropyl-β-cyclodextrin (degree of substitution, DS = 0.9) and another cyclodextrins were obtained from University of Berlin, Germany. Clenbuterol enantiomers was a gift from Thomae Pharmaceutical Company, Biberach an der Riss, Germany.

Buffer and sample preparation

Stock solution of 0.03 M HP-β-CD in 0.05 M borate/phosphate buffer solution was prepared by mixing an appropriate volume of sodium tetraborate solution with 0.05 M monobasic sodium phosphate solution, and adjusted to final pH values with 0.1 M phosphoric acid. The separation buffer was filtered and degassed prior to use. Stock solutions of racemic drug were prepared and stored at 4 °C. The water used throughout the experiments was ultrapure water from a E-pure TM system (Branstead, IOWA, USA).

Procedure

Prior to analysis, the fused silica capillary was purged for approximately 15 min with the separation buffer. Between runs, a flushing sequence consisting of 1 min with 0.01 M NaOH followed by 2 min with buffer was applied. The hydrostatic sample injection was accomplished by elevation of sample vial with respect to that buffer. The injection times were selected 3 and 5 seconds which correspond to volume of approximately 12 and 20 nL, respectively.

RESULTS AND DISCUSSION

Cyclodextrin-modified capillary electrophoresis

Since the main sources of separation modes depending on vectors point of electrophoretic mobility and electroosmotic flow in the same direction or not. The electroosmotic flow was determined using dimethylformamide or mesityl oxide as neutral marker (28). As clenbuterol enantiomers are basic compounds (pK_a = 10.2), they should be separated by applying electric fields with normal polarity (anode at the injection side) at acidic pH (pH = 2 - 6). Generally, the electrophoretic mobility of a CD-analyte complex is much lower than the mobility of the free (uncomplexed) molecule. The free CDs are uncharged and will move with the velocity of the electroosmotic flow. Consequently, separation of

enantiomers will occur, provided that the complexation constants of the optical isomers are different. The enantiomers and their CD complexes migrating toward the cathode are shown in Figure 1.

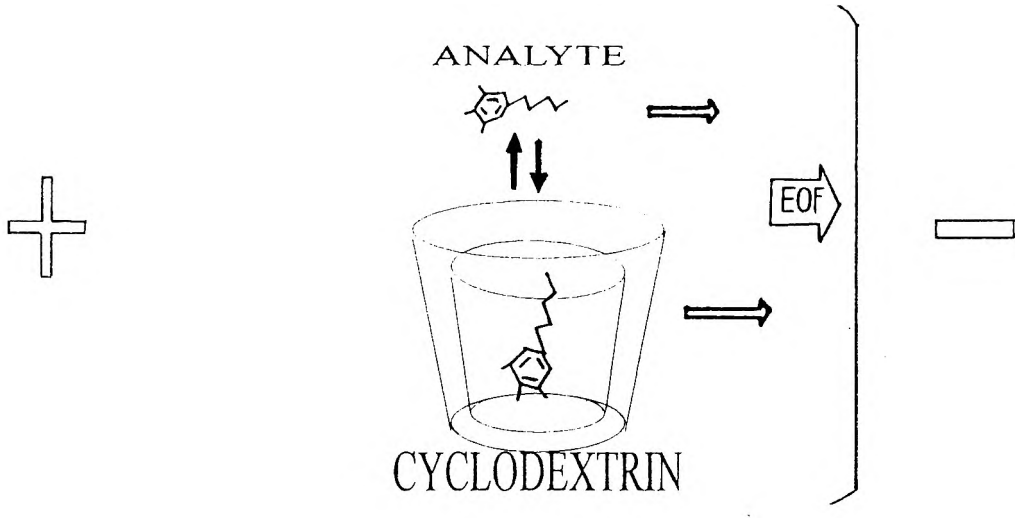

Figure 1 Separation mechanism in cyclodextrin-modified capillary electrophoresis.

Factors affecting chiral separations

Cyclodextrin type and concentration

Cyclodextrin is a cyclic glucopyranoses having a characteristic conical shape with a hydrophobic cavity and a polar exterior. Cyclodextrins and their alkylated derivatives are able to form inclusion complexes with many substances, including racemic drugs. The complex constants are determined by the size, geometry, hydrophobicity, and hydrogen-bonding ability of the analytes. Figure 2 shows the inclusion complexes model of clenbuterol enantiomers and HP-β-CD. Cyclodextrins can be used for chiral selector because of the enantioselective complexation involving the secondary hydroxyl groups on their larger circumference. Stable and selective complexation of the enantiomers with the CDs is important. Usually, separations can be achieved for structures of one aromatic ring (or similar size, and hydrophobicity) with β-CD. For substituted one-ring or two-ring structures the use of derivatized β-CD is suggested. Substituted two-ring, three ring or larger structures usually require the use of derivatized β-CD or γ-CD. It was found that the use of HP-β-CD was more informative than that of native β-CD as the former has greater solubility and could be studied over the full range of complexation.

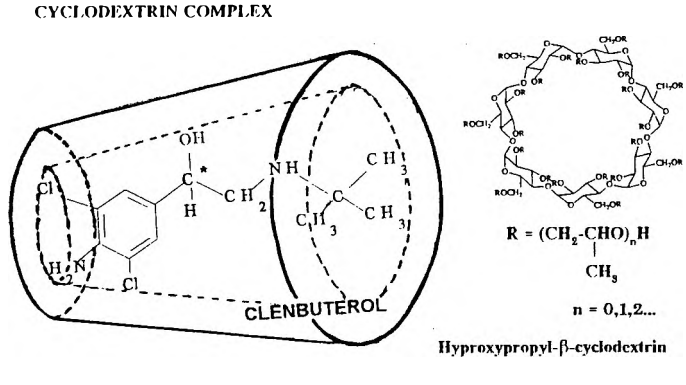

Figure 2 The inclusion complexes model of clenbuterol enantiomers and HP-β-CD.

The effect of cyclodextrin concentration on the separation of clenbuterol enantiomers is shown in Figure 3. Optimum separations were obtained at pH 5.0 and showed enantio-selectivity for separation of clenbuterol enantiomers up to concentration of 0.04 M, its limit of aqueous solubility. The observed migration times of clenbuteral enantiomers in the concentration range 0 - 0.04 M HP-β-CD exhibited a non-linear increase. This might be explained by the fact that high CD concentrations result in dimerization of cyclodextrins leading to a loss of selectivity(29). The concentration of chiral selector also affects the peak resolution and the migration time. Higher concentrations of cyclodextrins increase the viscosity of the buffer, thus resulting in increased migration of enantimers. Employing 0.05 M borate/phosphate buffer containing 0.03 M HP-β-CD provided a complete separation of clenbuterol enantimers with high resolution (Rs = 2.9) within 3.5 minutes (Figure 4).

pH of buffer solutions

The pH of the electrolyte buffer influences the ionic state at the capillary wall, thereby altering both electroosmotic flow (EOF) and solute-capillary wall interactions. As pH is reduced from 7 to 2, the value of EOF is significantly decreased. The ionic state of the chiral substances also influenced this efffect. Chiral separations of clenbuterol enantiomers were investigated separately between pH 2.2 to 7.0, optimum resolution was found at pH 5.0 where clenbuterol has a charge of +1.

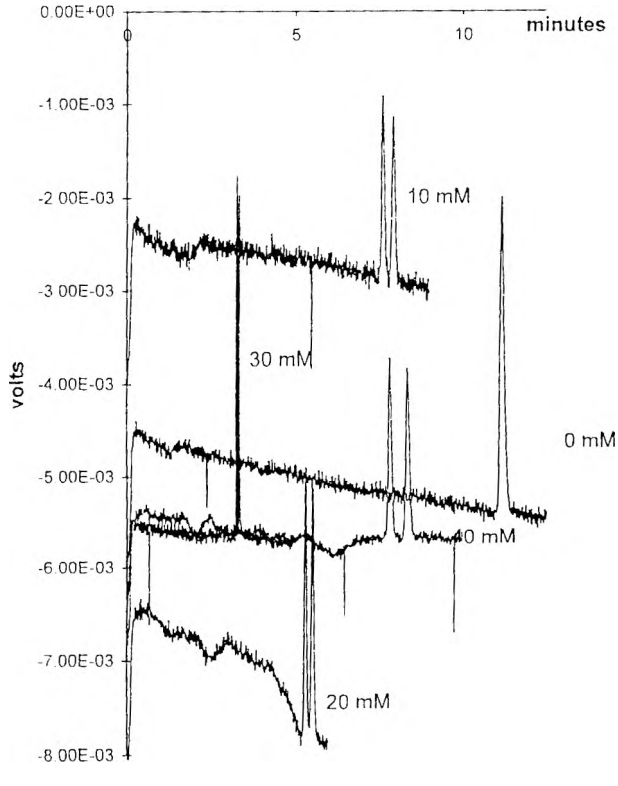

Figure 3 Impact of cyclodextrin concentration on the separation of clenbuterol enantiomers. Fused silica capillary: 32 X 24.5 cm, 50 μm I.D. Carrier electrolyte: 0 - 0.04 M HP-β-CD in 0.05 M borate/phosphate buffer, pH 5.0. Voltage +16 kV. UV detection at 214 nm. Sample: 0.15 mg/ml clenbuterol enantiomers. Vacuum sampling for 3 sec.

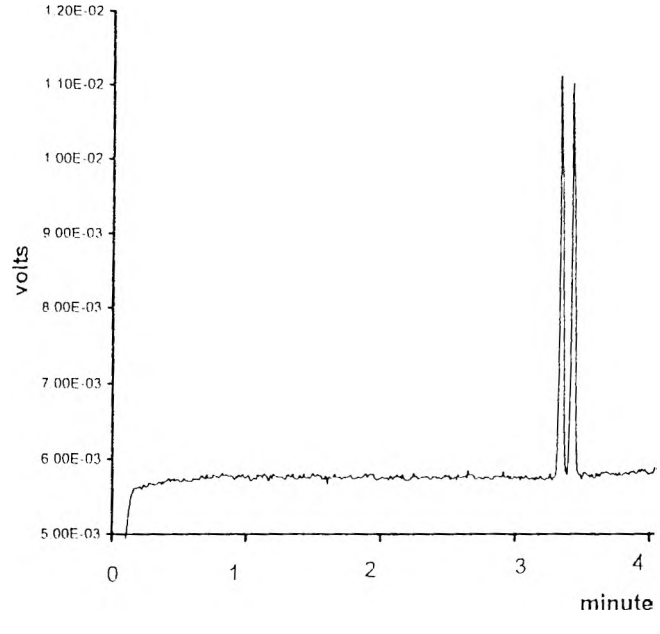

Figure 4 Chiral CE separation of clenbuterol enantiomers Fused silica capillary: 32 x 24.5 cm, 50 μm I.D Carrier electrolyte: 0.03 M HP-β-CD in 0.05 M borate/phosphate buffer, pH 5.0. Voltage +16 kV UV detection at 214 nm. Sample: 0.15 mg/m clenbuterol enantiomers. Vacuum sampling fc 5 sec.

Applied voltage and current

In CE, migration times can be shortened by increasing the applied voltage. Figure 5 shows the dependence of EOF and resolution values on the applied voltage. With a high voltage of about 650 V/cm, very short migration times are achieved but, owing to Joule heating and ineffective heat dissipation, an extreme decrease in selectivity results. The absolute rise in temperature at higher field strengths, however, will be significant and can be estimated to be ca. 5 ^{0}C thus accounting for a 10% increase in mobilities due to the lower bulk viscosity (30). Good resolution were obtained using a current of 100 μA, corresponding to an effective voltage of about 500 V/cm.

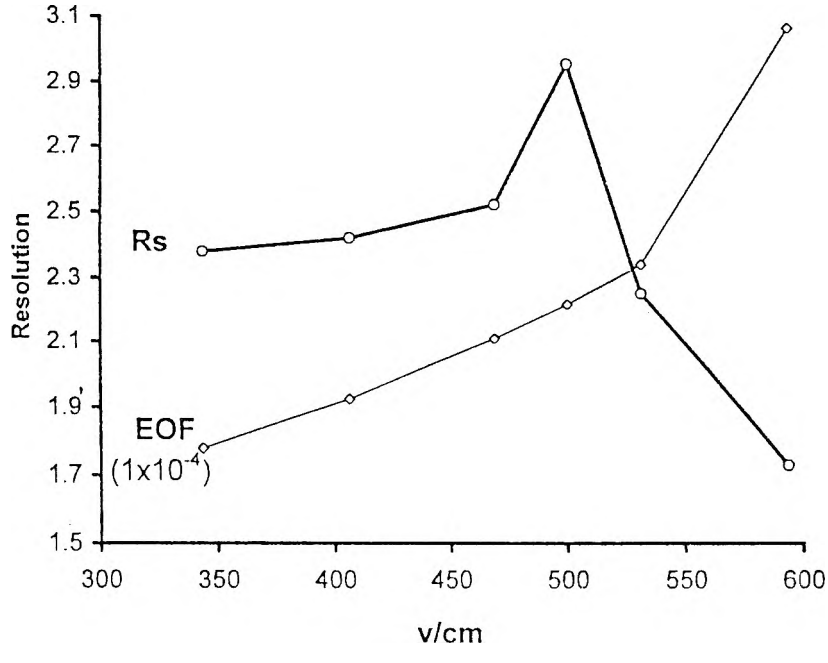

Figure 5 Impact of applied voltage on resolution and EOF. Fused silica capillary: 32 x 24.5 cm, 50 μm I.D. Carrier electrolyte: 0.03 M HP-β-CD in 0.05 M borate/phosphate buffer pH 5.0. Voltage +(16-19) kV. UV detection at 214 nm. Sample: 0.15 mg/ml clenbuterol enantiomers. Vacuum sampling for 3 sec.

Temperature

It is noted that the changes in the room temperature will make inconstant capillary temperature and affect the migration times(30). The increase in the selectivity values with a decrease in temperature might be explained by a decrease in rotational and/or vibrational energy, increasing the fixation of the enantiomers inside or at the top of the chiral selectors and, thus, increasing the enantioselectivity. For repeatability and good accuracy obtained, the temperature of the capillary was controlled at 25 ^{0}C throughout the experiments.

CONCLUSION

Cyclodextrin-modified CE has proved to be a powerful tool for resolving the enantiomers of pharmaceutical drugs. The optimum separation conditions depend on the type and concentration of chiral selectors, the pH of the buffer and temperature. The optimum run buffer conditions for the separa-

tion of clenbuterol enantiomers were found to be 0.03 M HP-β-CD in 0.05 M borate/phosphate buffer (pH 5.0) with an effective voltage of 500 V/cm at 25 ^{0}C.

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การแยกสารอิแนนทีโอเมอร์ของเคลนบิวเตอรอล โดยวิธีแคปิลารีอิเลคโทรโฟเรซิสที่ปรับเปลี่ยนด้วยซัยโคล เด็กซตริน

สุรพล นธการกิจกุล

ภาควิชาเภสัชเคมี คณะเภสัชศาสตร์ มหาวิทยาลัยเชียงใหม่

ได้รับ 20 กรกฎาคม 2540 ตอบรับ 9 ตุลาคม 2540

บทคัดย่อ : จากการศึกษาวิธีการแยกสารอิแนนทีโอเมอร์ของแคลนบิวเตอรอลโดยวิธีแคปิลารีอิเลคโทรโฟเรซิส ชนิดที่ปรับเปลี่ยนด้วยซัยโคลเด็กซตรินโดยมีไฮดรอกซีโปรพิล-บีต้า-ซัยโคลเด็กซตรินเป็นตัวเลือกชนิดไครอลพบว่าความสามารถในการแยกสารอิแนนทีโอเมอร์นี้ได้อย่างมีประสิทธิภาพและความรวดเร็วจะขึ้นกับการปรับสภาวะของค่าพารามิเตอร์ต่างๆ ได้แก่ ชนิดและความเข้มข้นของตัวเลือกชนิดไครอล การให้แรงคลื่นของสนามไฟฟ้า ความเป็นกรด-ด่างและบัฟเฟอร์ที่ใช้

กุญแจคำ: สารอิแนนทีโอเมอร์ของเคลนบิวเตอรอล,แคปิลารีอิเลคโทรโฟเรซิสที่ปรับเปลี่ยนด้วยซัยโคลเด็กซตริน