Enantioselective Extraction of Racemic Amlodipine Using Tartaric Acid Derivatives and β-Cyclodextrin Derivatives as Chiral Selectors

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Abstract: The distribution behavior of amlodipine enantiomers in a two-phase system containing tartaric acid derivatives in organic phase and beta-cyclodextrin (β-CD) derivatives in aqueous phase has been studied. The effect of extraction equilibrium time, influence of different alkyl chains of tartaric acids, types of beta-cyclodextrin derivatives, organic solvents and buffer pH were investigated. It was found that hydroxypropyl- β-cyclodextrin (HP-β-CD) has the strongest recognition ability among three β-CD derivatives of HP-β-CD, hydroxyethyl-β-cyclodextrin (HE-β-CD) and methylated-β-cyclodextrin (Me-β-CD) while D-diisopropyl tartrate has the strongest ability among four tartaric acid derivatives of L-diisopropyl tartrate, D-diisopropyl tartrate, L-diethyl tartrate and D-diethyl tartrate. The distribution coefficient and enantioselectivity, α gave an optimum value at pH 5.0.

Keywords: Chiral drug, Enantioselective extraction, Racemic amlodipine.

1. INTRODUCTION

Amlodipine, 3-ethyl 5-methyl-2-[(-2-(aminoethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate, is a racemic drug that belongs to the calcium channel blockers group, being used for treating hypertension and angina pectoris [1, 2]. It acts as a calcium antagonist inhibiting the membrane influx of calcium ions in vascular smooth muscles and cardiac muscles which in turn affects their contractile process and results in reduced blood pressure [3].

Today many drugs are used as a racemic mixture. All enantiomers may have different effects on the pharmacological activity, metabolism process and toxicity on human body [4, 5]. Like other chiral drugs, amlodipine consists of (*S*)-amlodipine and (*R*)-amlodipine. It has been reported that (*S*)-amlodipine shows a potent calcium channel blocker activity [6, 7] while the (*R*)-amlodipine has shown to release nitric oxide in the peripheral blood vessels which may lead to peripheral edema [8]. Hence, in order to reduce the incidence of peripheral edema and other side effects, it is beneficial to separate (*R*)-amlodipine from racemic (*R, S*)-amlodipine.

There are many techniques for separation of enantiomers such as crystallization, enzymatic conversion, chromatography and kinetic resolution [9]. These techniques are useful to separate the enantiomers of racemic drugs but there is still need for more efficient separation methods. Crystallization is the most used technique for separation of enantiomers but this technique requires many steps. Thus, it is time-consuming and cost-inefficient [9]. Enzymatic conversion is very expensive due to its single-action while chromatography is not suitable for production of large quantities of chiral substances. Solvent extraction is a good alternative method for chiral separation because it is usually preformed by dispersion of one immiscible phase in the other [10]. Moreover, it can be used in industrial scale which can be performed continuously with good effect and high recovery [5, 11].

The aim of the present study is to understand the distribution behavior of amlodipine enantiomers in a two-phase system containing tartaric acid derivatives in organic phase and beta-cyclodextrin derivatives in aqueous phase. The parameter studied includes the effect of extraction equilibrium time, influence of different alkyl chain of tartaric acids, types of beta-cyclodextrin derivatives, organic solvents and buffer pH.

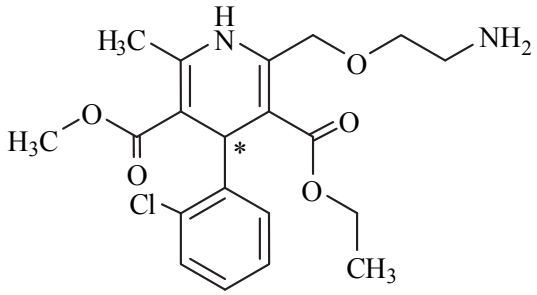

Fig. (1). Structure of amlodipine.

2. MATERIALS AND METHODS

2.1. Chemicals

Racemic amlodipine was purchased from MTT Pharma in China (>98%). β-CD derivatives and tartaric acid

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derivatives were obtained from Sigma Aldrich. Chemicals and solvents were purchased from Sigma Aldrich.

2.2. Preparation of Aqueous and Organic Solutions

An aqueous solution consist of 0.05 mmol/L (*R, S*)-amlodipine and 0.10 mol/L β-CD was prepared in 10 mmol/L acetate buffer. The pH was adjusted to pH 5.0. An organic phase consist of 0.20 mol/L tartaric acid derivatives which were dissolved in organic solvent.

2.3. Extraction Procedure

Batch extraction experiments were conducted by pipetting 3 ml of aqueous solution containing 0.05 mmol/L of (*R, S*)-amlodipine and 0.1 mol/L β-CD with 3 ml of the organic phase containing 0.20 mol/L tartaric acid derivatives, into 100 ml baffled flask. The flasks were then vigorously shaken in orbital shaker for the desired contact time to reach the maximum extract of (*R*)-amlodipine into the organic phase. The experiments were run at the temperature of 10ºC. The content of the flask was then transferred into a separatory funnel to separate the two phases. After the two phases were separated, the concentrations of (*R*) and (*S*)-amlodipine in aqueous phase were measured by HPLC.

The distribution coefficients of (*R*) and (*S*)-Amlodipine, k_R and k_S extracted from aqueous into organic phase were determined as Eq. (1) and Eq. (2);

$$k_R = \frac{\text{Concentration of (R)-Amlodipine in organic phase}}{\text{Concentration of (R)-Amlodipine in aqueous phase}} \quad \text{Eq. (1)}$$

$$k_S = \frac{\text{Concentration of (S)-Amlodipine in organic phase}}{\text{Concentration of (S)-Amlodipine in aqueous phase}} \quad \text{Eq. (2)}$$

The enantioselectivity (α) is defined as the ratio (k_R/k_S) of both distribution coefficients of (*R*)-amlodipine to (*S*)-amlodipine in an aqueous-organic two-phase system containing chiral selector in each phase as Eq. (3);

$$\alpha = \frac{k_R}{k_S} \quad \text{Eq. (3)}$$

2.4. Analytical Method

The concentration of (*R*) and (*S*)-amlodipine in aqueous phase was determined using a HPLC system (Shimadzu, Japan) equipped with a UV detector at the UV wavelength of 240nm. The standard curve was used to quantify the enantiomer. A 150mm x 4.0mm I.D. CHIRAL-AGP analytical column (ChromTech, Haegersten, Sweden) was used. The mobile phase was 10 mmol/L acetate buffer solutions (pH 4.5): 1-propanol (99:1, v/v) at a flow of 0.9 mL/min. The pH of the aqueous phase was measured with a pH meter (Fisher Scientific, MA, USA).

3. RESULTS AND DISCUSSION

3.1. Effect of Time of Chiral Extraction

In order to investigate the equilibrium time of extraction, 0.05 mmol/L (*R,S*)-amlodipine and 0.10 mol/L HP-β-CD in 10 mmol/L acetate buffer pH 5.0 were shaken in orbital shaker with 0.20 mol/L D-diisopropyl tartrate in decanol as organic solvent for the desired contact time. It was found that the chiral extraction reaches equilibrium after 6 hours.

3.2. Effect of Organic Solvents

Table **1** shows the influence of different organic solvents on distribution coefficient, *k* and enantioselectivity, α of amlodipine. The aqueous phase contains 0.05 mmol/L (*R,S*)-amlodipine in 10 mmol/L acetate buffer and 0.10 mol/L HP-β-CD. There is no chiral selector in organic phase. From the Table **1**, we can see the extraction performance of the three different kinds of organic solvents, alcohol > alkyl halide > hexane, which might be related with the polarity and interaction of different organic solvents with solute. For alcohol group listed in Table **1**, the enantioselectivity, α increased with the increase of the length of alcohol chain. It is seen from Table **1** that *n*-decanol is a suitable organic solvent for the extraction of racemic amlodipine.

Table 1. Effect of Organic Solvents on *k* and α

Organic Solvents	k_R	k_S	α
n-pentanol	3.56	3.65	0.98
n-hexanol	0.97	0.94	1.04
n-heptanol	0.53	0.50	1.05
n-octanol	0.26	0.24	1.08
n-decanol	0.42	0.28	1.47
1,2-dichloroethane	0.10	0.11	0.94
Dichloromethane	0.20	0.20	1.00
Hexane	0.02	0.03	0.66

3.3. Effect of β-CD Derivatives

A complex that formed between guest molecules and β-CD is depending on the size, shape and polarity of the guest molecule and various interactions involving electrostatic forces and hydrogen bonding. The size of the guest molecule determines whether it fits into the cavity, shape and polarity that influence the possible stabilizing effects by interactions within the cavity or with side groups on the cavity rim of β-CD [12]. The size of the guest molecule of amlodipine enantiomers fits into the cavity of β-CD derivatives. Therefore, β-CD derivatives can form complexes with amlodipine enantiomers. However the complexes that formed between amlodipine enantiomers and β-CD derivatives depend on the polarity of β-CD derivatives. So, three of the β-CD derivatives may show different enantioselectivities toward amlodipine enantiomers.

Table **2** shows that HP-β-CD has a higher distribution coefficient and high enantioselectivity as compared to HE-β-CD and Me-β-CD. The finding was similar to Mikus *et al.*, 2009 which studied the separation of amlodipine enantiomers using capillary electrophoresis [13]. It is also found from Table **2** that the value of k_S is less than k_R. It indicates that three β-CD derivatives recognize S-enantiomer, which means that β-CD forms complexes with (*S*)-amlodipine and is retained in the aqueous phase. HP-β-CD is chosen to be the most suitable chiral selector in aqueous phase among these three β-CDs derivatives.

Table 2. Effect of β-CD Derivatives

Types of β -CD	k_S	k_R	α
HP-β-CD	0.28	0.74	2.62
HE-β-CD	0.24	0.28	1.17
Me-β-CD	0.26	0.37	1.44

3.4. Effect of Tartaric Acid Derivatives

The distribution coefficient and enantioselectivity of amlodipine enantiomer were also determined in chiral extraction containing 0.10 mol/L HP-β-CD in aqueous phase and 0.20 mol/L tartaric acid derivatives in organic phase.

Table 3. Effect of Tartaric Acid Derivatives

Types of tartaric acid	k_S	k_R	α
L-diisopropyl	0.55	0.59	1.09
D-diisopropyl	2.61	3.00	1.15
L-diethyl	0.72	0.81	1.12
D-diethyl	0.93	1.06	1.13

Table **3** shows that the distribution coefficient of k_S and k_R increased when tartaric acid derivatives were added as a chiral selector in organic phase. The value of k_R for D-tartaric acid derivatives is larger than the value of k_R for L-tartaric acid derivatives. This indicates that D-tartaric acid derivatives preferentially recognize (*R*)-enantiomer. Table **3** shows that enantioselectivity of extraction increases with the addition of length alkyl chain of D-tartrate. Hence, the D-diisopropyl tartrate was chosen as the chiral selector in the organic phase because it has a high enantioselectivity among them.

3.5. Effect of Buffer pH

The pH is an important factor for consideration in the separation of enantiomers as it impacts the states of amlodipine enantiomers. The distribution coefficient and enantioselectivity of amlodipine enantiomers were studied with 0.20 mol/L D-diisopropyl tartrate in decanol solvent and 0.10 mol/L HP-β-CD in 10 mmol/L acetate buffer at different pH values.

Amlodipine has one distinct group which can be protonated to give an increase and decrease of pH, which is the amine group (Fig. **1**). In aqueous solution, amlodipine exists in two states of neutral molecule and anion. Therefore, there exists influence of pH on the distribution behavior of amlodipine enantiomers in enantioselective extraction. From the Fig. (**2**), the values of k_S decrease with the rise of pH until pH 5.0, and then it starts to increase at pH 5.5. For the values of k_R, it increases with the rise of pH but it decrease at pH 6.0.

From the Fig. (**3**), the enantioselectivity increases with the increase of pH until it reaches at pH 5.0 and then it starts to decrease. The possible reasons may be that the ratio between protonated and unprotonated amlodipine decreases with the rise of pH value. HP-β-CD and D-diisopropyl tartrate mainly have chiral recognition ability and affinity for molecular amlodipine but not for ionic amlodipine. Ionic amlodipine only exists in aqueous phase. At pH 5.0, the enantiomers of amlodipine are expected to be unprotonated. So, pH 5.0 was an appropriate choice in view of the bigger enantioselectivity of the enantioselective extraction.

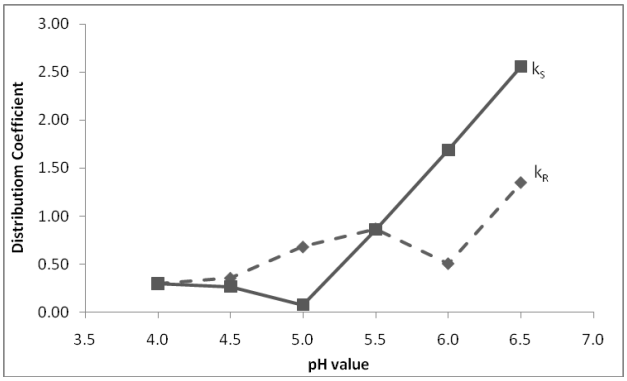

Fig. (2). Influence of pH on distribution coefficient, k_S and k_R. Organic phase: [D-diisopropyl tartrate] = 0.10 mol/L and [HP-β-CD] = 0.20 mol/L.

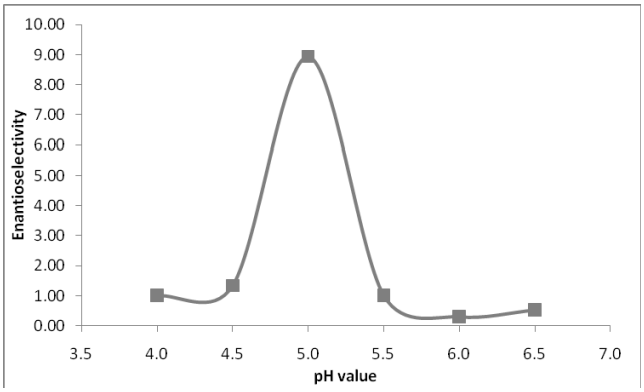

Fig. (3). Influence of pH on enantioselectivity. Organic phase: [D-diisopropyl tartrate] = 0.10 mol/L and [HP-β-CD] = 0.20 mol/L.

4. CONCLUSION

The present work has investigated the enantioselective extraction of racemic amlodipine using tartaric acid derivatives in organic phase and β-CD derivatives in aqueous phase as the chiral selectors. It was found that HP-β-CD has the strongest recognition ability among three β-CD derivatives of HP-β-CD, HE-β-CD and Me-β-CD while D-diisopropyl tartrate has the strongest ability among four tartaric acid derivatives of L-diisopropyl tartrate, D-diisopropyl tartrate, L-diethyl tartrate and D-diethyl tartrate. The enantioselectivity was optimum at pH 5.0.

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