Enantioselective Extraction of Ofloxacin Enantiomers Using Ester Alcohol *L*-Tartarate as Chiral Selector

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ABSTRACT: *The distribution behavior of ofloxacin enantiomers was examined in the aqueous and organic solvent of a two-phase system containing chiral selector L-tartarate. The effect of extraction equilibrium time, buffer pH, organic solvents and length of alkyl chain of L-tartarate on the partition coefficients and enantioselectivity of racemic ofloxacin were investigated, respectively. The L-tartarate formed more stable complexes with R-ofloxacin than that with S-ofloxacin. The partition coefficients and enantioselectivity of racemic ofloxacin increased with increasing length of alkyl chain of L-tartarate. Solvents showed a large influence on enantioselectivity and partition coefficients. Optimum buffer pH was about 7 for separation of racemic ofloxacin by extraction.*

KEY WORDS: *L-tartarate, Ofloxacin enantiomers, Chiral extraction, Partition coefficient, Enantioselectivity.*

INTRODUCTION

As the relation of chirality and activities of drugs is researched deeply, people become to realize the clinic importance of chirality. The different enantiomers of a drug can have vastly different pharmacological activities, pharmacokinetic processes and toxicity [1, 2]. The most well-documented example is that of the drug substance thalidomide. Bitter lessons and scientific research promote the interest in single-enantiomer drugs, so the potential of the chiral drug market is enormous [3]. How to obtain stereochemically pure drugs becomes one of the top-topics in the world. At present, chiral drugs represent above 50 % of the total number of sales, but nearly 85-90 % of them are racemic mixtures because of the difficulties of separation techniques. So preparative separations of enantiomers are very necessary [4].

To separate enantiomers, separation techniques such as crystallization, enzymatic conversion and chromatography have been developed. The techniques promote the research and development of chiral compounds, but there are some deficiencies about them. Crystallization is time- and cost-inefficient, and very often confined to such racemic compounds as acids and bases. Enzymatic conversion is expensive due to its single-action. As chiral stationary phases, mobile phases and derivatizing agents are very expensive, chromatography is cost-inefficient. To avoid the problems above, asymmetric synthesis and kinetic resolution have been developed [5-7].

However, it is very expensive and time-inefficient to develop a proper route for every chiral compound. Solvent extraction is a ripe separation technique of industries,

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the process of which can be easily performed automatically and continuously with good effect, high recovery and low energy consumption. Chiral solvent extraction is a very attractive option for separation of enantiomers [8, 9]. *S*-ofloxacin is one of the third best antibacterial agents which is highly regarded. In this paper, the distribution behavior of ofloxacin enantiomers was examined in the aqueous and organic solvent of a two-phase systems containing *L*-tartarate. As far as we know, there is no report on the enantioselective extraction of ofloxacin enantiomers using *L*-tartarate as chiral selector.

EXPERIMENTAL

Materials

Racemic ofloxacin was supplied by Kunshan Shanghe Pharmaceutical Firm in China and the purity is above 99.5 %. Other chemicals were of analytical regent grade.

Analytical method

Chromatographic studies were performed using a LC-10 AD pump (Shimadzu, Japan), an SIL-10 A injection valve with 20 µl loop, an SPD-10 A UV/VIS spectrophotometer detector (Shimadzu, Japan). An AT-130 temperature controller (Autoscience, Tianjin, PR, China) was used to control column temperature. A Kromasil RP-18, 5 µm, 4 mm × 250 mm column was used for the separation of ofloxacin enantiomers. Chromatographic conditions are the same as that in ref. [10]: 6 mmol/L *L*-phenylalanine and 3 mmol/L copper sulphate in water-methanol mobile phase (85: 15, v/v), flow-rate, 1 mL/min, detection at 300 nm (Fig. 1).

Enantioselectivity experiments

The enantioselectivity (a) is defined as the ratio (k_R / k_S) of both distribution coefficients of R-enantiomer to S-enantiomer in a aqueous-organic two-phase system containing chiral selector. In a 60 mL separatory funnel, 10 mL of an aqueous solution containing an aqueous 0.1 mol/L Na_2HPO_4/H_3PO_4 buffer and 0.2 g/L of RS-ofloxacin were shaken for some time with 10 mL of the organic phase containing 0.31 mol/L chiral selector at the temperature of 12 °C. After the two phases are separated, the concentrations of R- and S-enantiomer are measured by HPLC. From the results, k_R, k_S and a can be obtained.

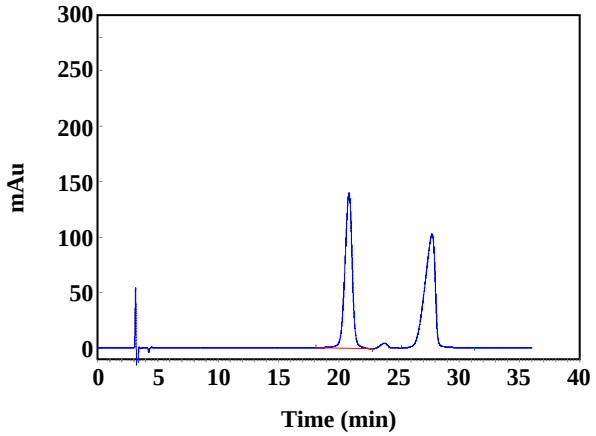

Fig. 1: HPLC chromatogram of rac OFLX.

THEORY

R- and *S*-enantiomers can form two diastereomeric complexes with *L*- or *D*- chiral selector through coulombic interactions, hydrogen bonding and van der Waals interaction in a chiral system containing *L* - or *D*-tartarate, which are described as follows:

$$R + L \Leftrightarrow R - L \quad (1)$$

$$S + L \Leftrightarrow S - L \quad (2)$$

$$R + D \Leftrightarrow R - D \quad (3)$$

$$S + D \Leftrightarrow S - D \quad (4)$$

The stability of the two complexes (*R-L* and *S-L* or *R-D* and *S-D*) is different in lipophilic organic phase, which can be represented by the difference in free energies of partition -Δ(Δ*G*). It can be deduced by the following equation

$$-\Delta(\Delta G) = -\Delta G_R - (-\Delta G_S) = RT\ln k_R - RT\ln k_S = RT\ln(k_R / k_S) = RT\ln\alpha \quad (5)$$

Only if a is not equal to 1, different degrees of separation of racemate can be achieved by chiral extraction.

RESULTS AND DISCUSSION

The effect of different alkyl chain of L-tartarate

Extraction of racemic mixtures is dependent on the difference in stability of the two diastereomeric complexes which are formed by chiral selector with the two enantiomer. So chiral extraction performance is related to the structure of chiral selector.

Table 1: Results of partition experiments with 0.31 mol/L solution of L-tartarate in 1,2-dichloroethane.

Ester alcohol moiety	k_R	k_S	α	-Δ(ΔG) / kJ.mol^{-1}
iso-propyl alcohol	2.98	2.57	1.16	0.35
iso-butyl alcohol	3.12	2.67	1.17	0.37
n-Butanol	3.26	2.79	1.17	0.37
iso-pentanol	3.37	2.86	1.18	0.39
n-pentanol	3.41	2.89	1.18	0.39
cyclohexanol	3.87	3.07	1.26	0.55
n-hexanol	3.60	3.03	1.19	0.41
n-heptanol	3.76	3.13	1.20	0.43
n-octanol	3.98	3.24	1.23	0.49
n-dodecanol	4.25	3.35	1.27	0.57
benzyl alcohol	3.52	3.01	1.17	0.37

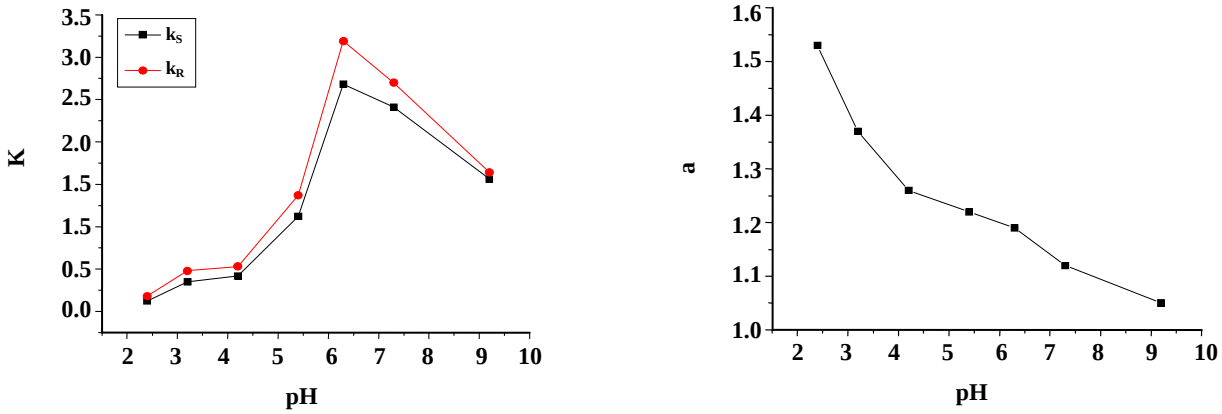

Fig. 2: Effect of buffer pH on distribution behavior. (a) Partition coefficients of ofloxacin isomers vary with pH; (b) selectivities between ofloxacin isomers in L-di-iso-pentyltartarate vary with pH.

It is very necessary to study the influence of different alkyl chain of *L*-tartarate on k and *α* of both ofloxacin enantiomer,with the chiral selector concentration of 0.31 mol/L, 0.2 g/L ofloxacin, 1, 2-chloroethane as organic solvent and 0.1 mol/L Na_2HPO_4/H_3PO_4 buffer at pH 6.86. It is seen from table 1 that k_R is always bigger than k_S, that is to say, the complexes of *L*-tartarate with oflocaxin *R* are more stable than that of *L*-tartarate with oflocaxin *S*. The partition coefficients and enantioselectivity increase with the addition of length of alkyl chain of *L*-tartarate.

The effect of buffer pH

pH has been reported to be a parameter affecting the resolution in a chiral extraction system [11]. To investigate the effect of buffer pH on k and α, distribution behaviors of ofloxacin enantiomers are examined in the aqueous and organic solvent of a two-phase system containing 0.31 mol/L *L-iso*-pentyltartarate, 0.1 mol/L Na_2HPO_4/H_3PO_4 buffer, 0.2 g/L of ofloxacin and 1, 2-chloroethane as organic solvent at different pH. The results obtained are shown in Fig. 2.

The k increases obviously with the increase of buffer pH firstly. Then begin to decrease when pH is above 7. However, the *α* decreases with the increase of buffer pH. So it was a appropriate choice of pH such as 7 in view of the bigger k and α in favor of the chiral separation.

The effect of organic solvent

Chiral extraction performance is not only related to the structure of chiral selector, but also to the properties

Table 2: Influence of organic solvents on k, α and -Δ(ΔG).

Organic solvent	k_R	k_S	α	$-\Delta(\Delta G)$ /kJ.mol^{-1}
1,2-dichloroethan	3.37	2.86	1.18	0.39
n-heptane	0.16	0.14	1.14	0.31
n-decanol	0.35	0.29	1.21	0.45
n-nonanol	0.29	0.25	1.16	0.35
n-octanol	0.26	0.22	1.18	0.39
n-heptanol	0.24	0.20	1.20	0.43
n-hexanol	0.21	0.18	1.17	0.37
n-pentanol	0.17	0.15	1.13	0.29
iso-pentanol	0.16	0.15	1.07	0.16
n-butanol	0.13	0.12	1.08	0.18

of organic solvents. So it is very important to investigate the influence of different organic solvent on k and α of ofloxacin enantiomers, containing 0.1 mol/L Na_2HPO_4/ H_3PO_4 buffer, 0.31 mol/L *L*-iso-pentyltartrate, and 0.2 g/L of ofloxacin at pH 6.86. From table 2, we can see that the extraction performance for the three kinds of organic solvents is different, for example, 1, 2-dichloroethane > alcohol > heptane, which might be related with the polarity and interacts of different organic solvent with solute. The partition coefficients and enantioselectivity generally increase with the addition of length of alkyl chain of alcohol.

The effect of time of chiral extraction

During the course of the chiral extraction, the reversible reactions that *L*-tartrate form diastereomeric complexes with *R*- and *S*-ofloxacin by non-covalent bonding, are involved. In general, the reversible reactions are slow. Therefore, it is necessary to investigate the equilibrium time to evaluate the separation ability of chiral extraction containing 0.31 mol/L *L-iso*-pentyltaratrate, 0.1 mol/L Na_2HPO_4/H_3PO_4 buffer at pH 6.86, 0.2 g/L of ofloxacin and 1, 2-chloroethane as organic solvent. It is found that the chiral extraction reaches equilibrium after 1 h.

CONCLUSIONS

the *L*-tartarate form more stable complexe with *R*-ofloxacin than that with *S*-ofloxacin, the partition coefficients and enantioselectivity of racemic ofloxacin increase with addition of length of alkyl chain of *L*-tartarate, solvents have a large influence on enantio-selectivity and partition coefficients, optimum pH is about 7 for separation of racemic ofloxacin by chiral extraction.

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