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Short Communication

Resolution of terfenadine enantiomers by reversed phase-high performance liquid chromatography using β-cyclodextrin as mobile phase additive

Sekhar Surapaneni[a,b,*], Shoukry K.W. Khalil[a]

[a]*Department of Pharmaceutical Sciences, North Dakota State University, Fargo, ND 58105, USA*
[b]*Drug Metabolism and disposition, Drop Code 0825, Eli Lilly and Company, Indianapolis, IN 46285, USA*

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1. Introduction

Terfenadine is a non-sedating H1-receptor antagonist used in the treatment of allergic rhinitis [1]. Structurally, terfenadine has an asymmetric carbon and is administered as the racemate. It undergoes extensive first-pass metabolism (over 99% of the absorbed dose) resulting in two metabolites [2,3]. Fig. 1 shows the structures of terfenadine and its active acid metabolite. Recently it has been shown that terfenadine undergoes stereoselective metabolism [4,5]. Few high performance liquid chromatographic (HPLC) procedures for the separation of terfenadine enantiomers have been described [6–8]. All of these reported methods use chiral stationary phases for separation of enantiomers. Cyclodextrins have been used as chiral stationary phase or mobile phase additive for stereoselective resolutions in HPLC [9–12]. Cyclodextrins, as mobile phase additive, provede greater resolution than cyclodextrin columns. This paper describes the enantioselective resolution of terfenadine by reversed phase HPLC using β-cyclodextrin (β-CD) as mobile phase additive. The stoichiometry of complexation of terfenandine with β-CD is also investigated. The effect of β-CD concentration and ethanol concentration on resolution is described. The stoichiometry of complexation of terfenadine with β-CD and the effect of functional group in the separation process are investigated. The method provides a cheaper and more rugged alternative because the chromatography is performed on a conventional reverse phase column.

2. Experimental

2.1. Materials and reagents

Racemic terfenadine was obtained from KV Pharmaceuticals. Acetonitrile, methanol, and diethylamine were purchased from Fisher (Fail Lawn, NJ). Ethanol (200 proof) was obtained

* Corresponding author. Tel.: (+1) 317 277 6702.

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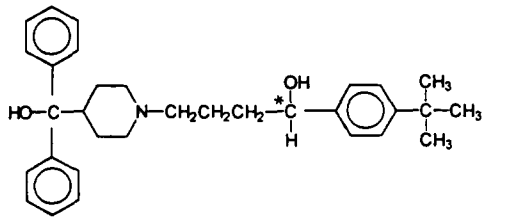
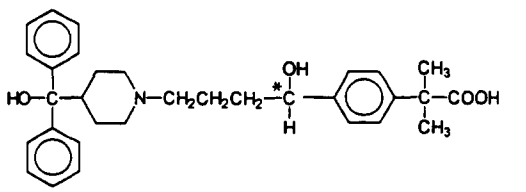


TERFENADINE



ACID METABOLITE

Fig. 1. Structure of terfenadine and its acid metabolite (the asterisk indicates an asymmetric center).

from Quantum Chemical Corporation. Sodium perchlorate and β-CD were purchased from Sigma (St. Louis, MO). All other chemicals were reagent grade.

2.2. Instrumentation

The chromatographic system consisted of a Hewlett-Packard model 1050 liquid chromatograph equipped with a model 1050 HP variable-wavelength UV detector, an autosampler, and a 3392A HP recorder. The chromatography was carried out using a Microsorb 5 μm ODS (25 cm $\times$ 406 mm i.d.) column (Rainin, USA). The flow rate was 0.5 ml min^{-1} and the eluent was monitored at 205 nm.

2.3. Stock solutions

Stock solutions (200 μg ml^{-1}) of terfenadine and acid metabolite were prepared in methanol. The solutions were stored at 4°C. The working solutions (10 μg ml^{-1}) were prepared in the mobile phase and 10 μl was injected.

2.4. Mobile phase

Sodium perchlorate buffer 0.1 M was prepared in deionized distilled water. The mobile phase consisted of buffer:ethanol:diethylamine (70:30:0.5% v/v) with a 35.24 mM concentration of β-CD. The final pH of the mobile phase was adjusted to 6.4.

2.5. Spectral analysis of the enantiomers

Ultraviolet–visible absorption spectra of samples in methanol were determined using a 1 cm pathlength quartz cuvette with a Beckman DU-64 model spectrophotometer. Circular dichroism spectra of samples in methanol in a quartz cell of 1 cm pathlength were recorded using a Jasco model 500A spectrophotometer equipped with a model DP-500 data processor. The circular dichroism spectrum is expressed in terms of ellipticity (in millidegrees). The optical rotation was measured using a Jasco DIP-370 digital polarimeter with a 100 mm cell. Chiral chromatography was carried out repetitively to collect sufficient amounts of terfenadine enantiomers for the determination of absolute configuration. The solutes were evaporated to dryness and dissolved in methanol for spectral analysis.

3. Results and discussion

The stereochemical assignment of the enantiomers from the column was based on circular dichroism spectral analysis. The first eluate from chiral chromatography was found to be the (+)-enantiomer and was assigned an absolute configuration of *R*, based on previous reports [6,7]. UV–Vis spectra of both enantiomers were identical. The measurement of optical rotations of enantiomers confirmed the results of circular dichroism spectra. Acetonitrile, methanol, and ethanol were tried as organic modifiers in the mobile phase. Addition of acetonitrile or methanol to buffer permitted limited solubility of β-CD in the mobile phase. However, addition of ethanol to the buffer allowed the use of high concentrations of β-CD. As a result ethanol was

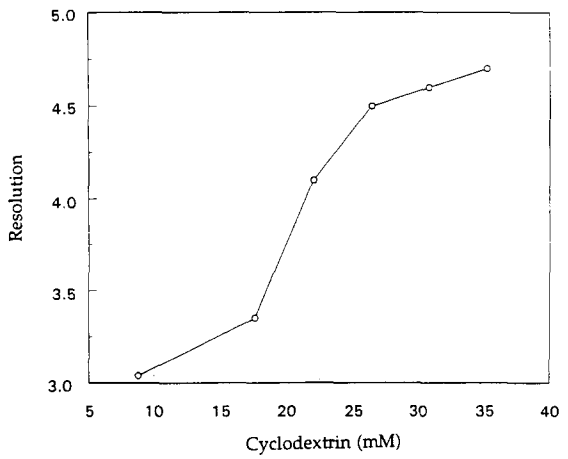


Fig. 2. Effect of β-CD concentration on resolution.

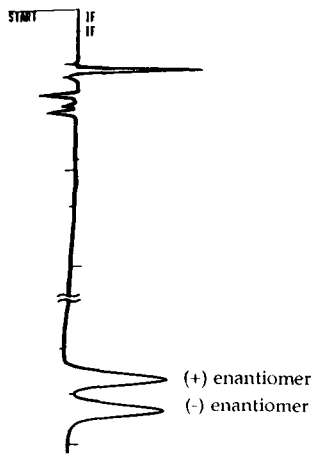


Fig. 3. Chromatogram showing enantiomeric resolution of terfenadine: (+) *R*-enantiomer 43 min, (−) *S*-enantiomer 46.5 min.

chosen as the organic modifier in this study. In the absence of ethanol from the mobile phase, complexes of β-CD and terfenadine eluted after 180 min. As the concentration of ethanol increased, the capacity factors decreased significantly because of decreased interaction between the inclusion complexes and the stationary phase. As a result the peak shape improved. However, increasing the ethanol concentration decreased the selectivity factor (α) and the resolution between the two enantiomers diminished. Optimum separation was achieved with 30% ethanol concentration. The effect of flow rate on resolution was also studied. Increasing the flow rate resulted in decreased capacity factors but it also diminished the resolution. An optimum resolution was achieved with a flow rate of 0.5 ml min^{-1}.

The effect of β-CD concentration in the mobile phase on the resolution of the enantiomers is shown in Fig. 2. At a low concentration of β-CD the resolution was partial. Increasing the concentration of β-CD increased the resolution, which reached a plateau at higher concentrations. Also, increasing the β-CD concentration resulted in decreased capacity factors. The baseline separation was achieved with a 35.24 mM β-CD concentration in the mobile phase (Fig. 3).

In most cases it is assumed that complexation between β-CD and guest molecule is 1:1. In some cases two or more CD molecules can bind to a single quest molecule. Armstrong et al. [13] derived the following equation describing the relationship between capacity factor and CD concentration in the case of 2:1 stoichiometry:

$$\frac{1}{k'} = \frac{1}{\phi k[A]} + \frac{k_1[CD]}{\phi k[A]} + \frac{k_1 k_2[CD]^2}{\phi k[A]}$$

where k, k_1, k_2 are equilibrium constants, ϕ is the phase ratio, and A is a stationary phase absorption site. A plot of $1/k'$ vs. [CD] resulted in a vurve (Fig. 4). This is indicative of the behavior of a solute that binds more than one cyclodextrin. A plot of $1/k'$ vs. $[CD]^2$ resulted in a linear plot at

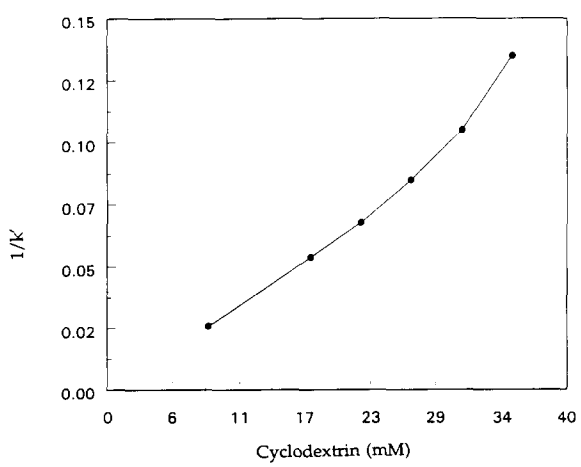


Fig. 4. Plot of $1/k'$ vs. β-CD concentration.

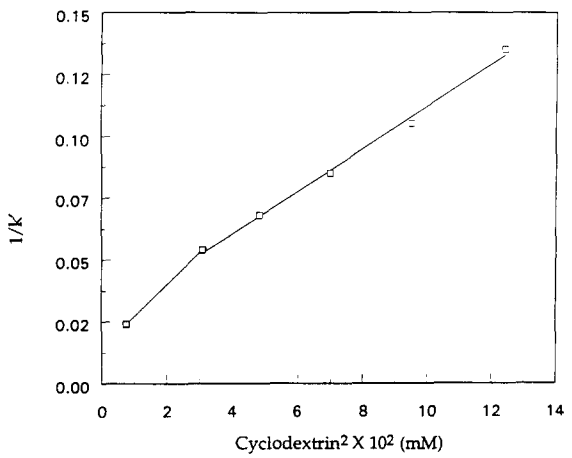


Fig. 5. Plot of $1/k'$ vs. square of β-CD concentration.

moderate to high concentrations of cyclodextrin (Fig. 5). This suggests that β-CD forms 2:1 complexes with terfenadine in the separation process. Terfenadine has a hydrophobic terminal group and a hydroxy functional on the chiral carbon to form an inclusion complex with the cavity and to hydrogen bond with the mouth of β-CD respectively. To test whether the terminal tertiary butylphenyl group is involved in complexation, chromatography was carried out on racemic acid metabolite under similar mobile phase conditions as for terfenadine. The resulting chromatogram (Fig. 6) showed no separation of enantiomers. The polar carboxylic group prevents the metabolite from interacting with the cavity of β-CD. This suggests that the tertiary butylphenyl group is

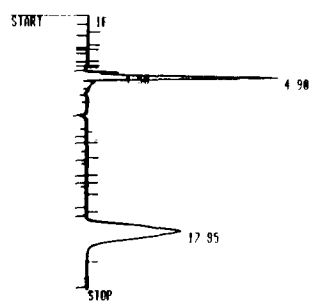


Fig. 6. Chromatogram of racemic acid metabolite. *R*- and *S*-enantiomers coelute (17.5 min).

essential for chiral recognition. A plot of $1/k'$ vs. [CD] with acid metabolite showed a linear relationship indicating that there is 1:1 complexation with β-CD. This suggests that β-CD in some manner interacts with hydroxy diphenyl moiety. This interaction is not essential for the separation of enantiomers.

4. Conclusions

Complete baseline separation of terfenadine enantiomers was achieved by reverse phase HPLC using β-CD as mobile phase additive. Studies with terfenadine acid metabolite indicated that tertiary butyl phenyl group was essential in chiral recognition and that the hydroxy diphenyl moiety was involved in complexation but has no bearing on stereoselective resolution. It appears from the studies that β-CD forms 2:1 complexes with terfenadine in the separation process.

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