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Enantioseparation and impurity determination of the enantiomers of novel phenylethanolamine derivatives by high performance liquid chromatography on amylose stationary phase

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ABSTRACT

Simple and efficient analytical HPLC methods using Chiralpak AS-H as chiral stationary phase were developed for direct enantioseparation of 11 novel phenylethanolamine derivatives. The chromatographic experiments were performed in normal phase mode with *n*-hexane–ethanol–triethylamine (TEA) as mobile phase. Excellent baseline enantioseparation was obtained for most of compounds. The effects of the concentration of organic modifiers and column temperature were studied for the enantiomeric separation. The mechanism of chiral recognition was discussed based on the relationship between the thermodynamic parameters and structures of compounds. It was found that the enantioseparations were all enthalpy driven, and the *tert*-butyl groups of compounds had significant influence on the chiral recognition. Trantinterol enantiomers were resolved ($R_s = 2.73$) within 14 min using *n*-hexane–ethanol–TEA (98:2:0.1, v/v/v) as mobile phase with a flow rate of 0.8 mL min^{-1} at 30 °C. The optimized method was validated for linearity, precision, accuracy and stability in solution and proved to be robust. The limits of detection (LOD) and quantification (LOQ) for (+)-trantinterol were 0.15 and 0.46 μg mL^{-1}. The method was applied for enantiomeric impurity determination of (−)-trantinterol bulk samples.

1. Introduction

Over the past century β_2-adrenoceptor agonists have evolved to become established as one of the front-line treatments for the respiratory conditions asthma and chronic obstructive pulmonary disease. The 1-phenyl-2-aminoethanol hydrochlorides, such as mabuterol, bambuterol and terbutaline, are representative β_2-adrenoceptor agonists and have been used for the treatment of asthma in clinics. Recently, we synthesized a set of novel phenylethanolamine derivatives, 2-phenyl-2-aminoethanol hydrochlorides, on the principle of isosterism with 1-phenyl-2-aminoethanol hydrochlorides. Preliminary pharmacological studies revealed that a number of them showed potential trachea relaxing activity, and most of the (−)-enantiomers were more potent than corresponding (+)-enantiomers. Thus, it is necessary to develop chiral separation methods for these novel β_2 agonists for further study. Trantinterol (compound 2), 2-(4-amino-3-chloro-5-trifluoromethylphenyl)-2-*tert*-butylamino-ethanol hydrochloride, one of the above compounds, is a novel β_2-adrenoceptor agonist which

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presents both potent trachea relaxing activity and high β_2 selectivity with low cardiac side effect [1]. It is currently undergoing phase II clinical trials in China for the treatment of asthma. Moreover (−)-trantinterol exhibited more potent efficacy, higher affinity and better selectivity for β_2-adrenoceptor than (±)- and (+)-trantinterol [2]. Therefore, it is important to establish an enantioseparation method for trantinterol to quantitate the enantiomers. The major separation technique for the enantioseparation and determination of chiral purity is high performance liquid chromatography (HPLC) with chiral stationary phase (CSP) [3–8].

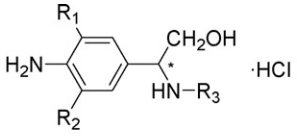
A survey of literature showed that no chiral chromatography method has been used in the enantioseparation of phenylethanolamine derivatives on Chiralpak AS-H column.

In this paper, we developed simple and efficient HPLC methods with Chiralpak AS-H as CSP for direct separation of 11 newly synthesized phenylethanolamine derivatives and validated the method for enantiomeric impurity determination of (−)-trantinterol.

2. Experimental

2.1. Chemicals and reagents

Compounds 1–11 were synthesized in our laboratory leading to racemic mixtures of enantiomers (chemical purity >99.5%), and their chemical structures are given in Fig. 1. (−)-Trantinterol and (+)-trantinterol (enantiomeric purities > 99.97%) were isolated by twice resolution with L-tartaric acid from (±)-trantinterol. *n*-Hexane and ethanol of HPLC grade were purchased from Concord Technology Co. Ltd. (Tianjin, China). Analytical grade triethylamine was purchased from Bodi Chemicals Co. Ltd. (Tianjin, China).



compound	R_1	R_2	R_3
1	Cl	CF_3	$CH(CH_3)_2$
2	Cl	CF_3	$C(CH_3)_3$
3	Cl	CF_3	
4	Cl	CF_3	
5	Cl	CF_3	$CH_2CH_2CH_2CH_3$
6	Br	CF_3	$CH(CH_3)_2$
7	Br	CF_3	$C(CH_3)_3$
8	Cl	Cl	$CH(CH_3)_2$
9	Cl	Cl	$C(CH_3)_3$
10	Br	Br	$CH(CH_3)_2$
11	Br	Br	$C(CH_3)_3$

Fig. 1 – The chemical structures of chiral phenylethanolamine derivatives studied.

2.2. Apparatus

All enantioseparations were carried out on a Shimadzu HPLC system (Kyoto, Japan) equipped with an SPD-10A UV–vis detector and LC-10AT pump. Data acquisition was performed using a Sepu3000 Chromatography Data System obtained from Puhui Technology Co. Ltd. (Hangzhou, China).

2.3. Sample preparation

Analytical solutions of racemic compounds 1–11 of 300 $\mu g\,mL^{-1}$ were prepared by dissolving the appropriate amount of the substances in a mixture of *n*-hexane and ethanol in a proportion of 80:20. Stock solutions of (+)-trantinterol (500 $\mu g\,mL^{-1}$) and (−)-trantinterol (2 $mg\,mL^{-1}$) were prepared by dissolving the appropriate amount of the substances in a mixture of *n*-hexane and ethanol in a proportion of 80:20.

2.4. Chromatographic conditions

The chromatographic conditions were optimized using an amylose-based chiral stationary phase Chiralpak AS-H (250 mm × 4.6 mm i.d., 5 μm, Daicel Chemical Industries Ltd., Tokyo, Japan) which was safeguarded with Chiralpak AS-H (10 mm × 4.0 mm) guard column. Mixtures of *n*-hexane and ethanol in different ratios were investigated as mobile phase. The mobile phase used for method validation was *n*-hexane–ethanol–TEA (98:2:0.1, v/v/v). A flow rate of 0.8 $mL\,min^{-1}$ was used. The detection was carried out at a wavelength of 254 nm. The injection volume was 5 μL. The column temperature was 30 °C unless noted in the study of temperature dependence of the enantiomeric resolution.

2.5. Method validation for quantification of (+)-trantinterol in (−)-trantinterol

2.5.1. Linearity

Linearity of (+)-trantinterol was determined by using seven calibration solutions in the concentration range of 0.5–15 $\mu g\,mL^{-1}$ (0.5, 1, 3, 6, 9, 12, 15 $\mu g\,mL^{-1}$), prepared in *n*-hexane–ethanol (80:20, v/v) from stock solution. Each concentration was injected in triplicate. The linear regression analysis was made by plotting average peak area versus analyte concentration.

2.5.2. Limits of detection (LOD) and quantification (LOQ)

LOD and LOQ of (+)-trantinterol were determined by calibration curve method [9] according to the following equations:

$$\mathrm{LOD} = \frac{3.3\sigma}{S} \qquad \mathrm{LOQ} = \frac{10\sigma}{S}$$

where σ and S are the standard deviation of the response and the slope of the calibration graph, respectively.

2.5.3. Precision

The precision of method was determined as repeatability, intra-day and inter-day precision. The results were expressed in terms of relative standard deviation (R.S.D.%) values for