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Diastereomeric and enantiomeric high-performance liquid chromatographic separation of synthetic anisodamine

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Abstract

In order to investigate the enantiomeric pharmacokinetics and biotransformation of synthetic anisodamine (654-2), a cholinoceptor antagonist widely used in clinic in China, it has been preparatively separated into two racemates (I and II) by using ZORBAX Eclipse XDB-C18 column. The diastereo- and/or enantioseparations of 654-2, I and II were carried out by HPLC using CHIRALPAK AD-H as chiral stationary phase (CSP) and acetonitrile–2-propanol–DEA 97:3:0.1 (v/v/v) as mobile phase. The methods were optimized by studying mobile phase modifiers, concentration of modifier and column temperature. The HPLC method for the simultaneous separation of two pairs of enantiomers of 654-2 has been validated.

Keywords: Enantioselective HPLC; Chiral stationary phases; Diastereoisomers; Anisodamine; Tropane alkaloids

1. Introduction

Anisodamine (654-1), a tropane alkaloid isolated from the Chinese solanaceous plant (*Scopolia tangutica* Maxim.) [1], is a potent cholinoceptor antagonist and has been used as a spasmolytic drug in China for decades by virtue of its weaker side effect on the central nervous system than atropine [2]. Furthermore, anisodamine was demonstrated to inhibit thrombogenesis, granulocyte and platelet aggregation and has been used in the treatment of acute microcirculatory disturbances caused by infections, such as fulminant epidemic meningitis, toxic bacillary dysentery, septic shock, severe lobar pneumonia and hemorrhagic enteritis [3,4]. The chemical structure of anisodamine is (2*S*)-3-hydroxy-2-phenyl-propionic acid (3*S*,6*S*)-6-hydroxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester. Because of the increased demand in clinics and the limited amounts of anisodamine available from the natural resources, now its synthetic one (trade name 654-2), a racemic mixture of two pairs of enantiomers [5,6] shown in Fig. 1, is massively used in China.

The biological properties of this kind of alkaloids including cholinoceptor agonists [7–9] and antagonists, like most chiral drugs, depend strongly on their stereochemistry [10]. The clinical studies indicated that the spasmolysis of 654-2 was almost identical with that of 654-1, but the side effect of 654-2 was stronger than that of 654-1 [11]. Evidently, the therapeutic and side effects of 654-2 were the combination effect of four isomers. The potency differences among four isomers of 654-2 on muscarinic receptor have been observed [12]. The optimization of pharmacological properties of 654-2 warranted our evaluating the enantioselective pharmacokinetics of 654-2 and its each pair of enantiomers. Thus, there is a clear need for method able to obtain two racemates (I and II) and analytical methods by which enantioseparation of 654-2, I and II can be realized. Although certain capillary electrophoresis (CE) methods with high resolution capability and short analysis time have been established for the enantioseparation of 654-2 [5,6,13–15], no HPLC method, one of the most widely used techniques for the enantioselective analysis of chiral drugs [16], has been reported for the enantioseparation of 654-2. In this paper, we report the preparative diastereo-separation of I and II from 654-2 by the reverse phase HPLC and the direct enantioseparation of 654-2, I and II on HPLC with CHIRALPAK AD-H. Our chiral HPLC method with the possibility to transfer to preparative scale

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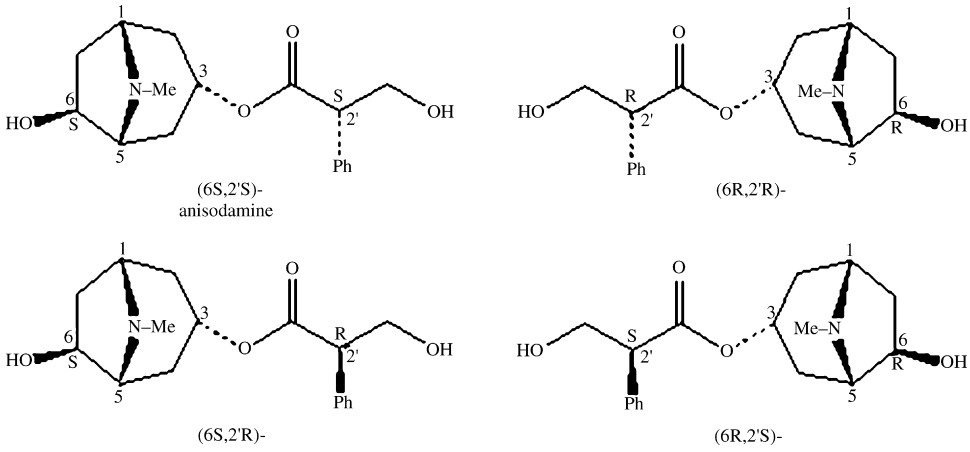

Fig. 1. Structure of four stereoisomers of 654-2.

provides an alternative for the separation of four stereoisomers of 654-2.

2. Experimental

2.1. Chemicals and materials

654-2, a mixture of (6*S*,2′*S*)-, (6*S*,2′*R*)-, (6*R*,2′*R*)- and (6*R*,2′*S*)-isomers, was provided by Shanghai No.1 Biochem & Pharm Company (Shanghai, China). HPLC grade *n*-hexane, 2-propanol and methanol were purchased from Dikma (Beijing, China), and acetonitrile from Sigma–Aldrich (St. Louis, USA). Diethylamine (DEA) was obtained from Shanghai Reagent (Shanghai, China).

2.2. Equipments

Chromatographic studies were performed on Agilent 1100 HPLCs (Agilent, Palo Alto, CA, USA), equipped with an autosampler, thermostat-column device, a variable-wavelength UV detector operating at 235 nm and a data acquisition system using the HP Chemstation software. The ^{1}H NMR spectra were measured by using Bruker AV 500 apparatus.

2.3. Chromatographic conditions

The preparative separation was achieved on ZORBAX Eclipse XDB-C18 column (250 mm × 9.4 mm i.d., packed with 5 μm diameter particles, Agilent Technologies, MN, USA) using methanol–water (53:47, v/v) containing 1.7% DEA as a mobile phase with the flow-rate 2.0 ml/min (Fig. 2). Injection volume was 30 μl.

The enantioseparation was achieved on CHIRALPAK AD-H column (250 mm × 4.6 mm i.d., particle size 5 μm) from Daicel Chemical Industries (Tokyo, Japan) using the eluents consisting of a mixture of *n*-hexane–2-propanol (92:8, v/v) or acetonitrile–2-propanol (97:3, v/v), with the addition of 0.1 vol. of DEA, with the flow-rate of 0.9 ml/min. All separations were performed at 25 °C, except those used for the study of the effect of temperature on HPLC. CHIRALPAK AD-RH column

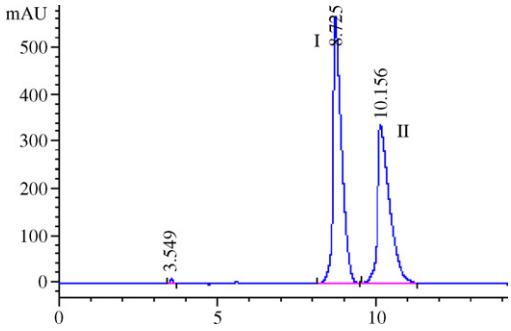

Fig. 2. Separation of diastereomers of 654-2 on ZORBAX Eclipse XDB-C18 column. Eluent: methanol–deionized water–DEA (53:47:0.017, v/v/v).

(150 mm × 4.6 mm i.d., particle size 5 μm) from Daicel was employed during method development.

2.4. Validation of the method

Detector response linearity was checked by preparing calibration sample solutions of 654-2 at concentration range of 65–1300 μg/ml using the selected mobile phase as the solvent. Linear regression curve was obtained by plotting peak area versus concentration, using the least squares method.

The method precision was assessed using three standard solutions of 654-2 at 130, 260 and 520 μg/ml. The accuracy of the method was evaluated by back-calculation. Limits of detection (LOD) were established at a signal-to-noise ratio of 3 and limits of quantification (LOQ) at a signal-to-noise ratio of 10.

3. Results and discussion

3.1. Preparative separation of diastereomers from 654-2

The preparative separation of diastereomers from 654-2 was carried out by using ZORBAX Eclipse XDB-C18 column. The capacity factor k'_1 of I, separation factor α and resolution R_s were 1.46, 1.27, 2.56, respectively, which were sufficient for the preparative separation. Two racemates (57.1 mg I and 46.4 mg II) eluted successively were collected from 654-2 (125 mg). I