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Reversed-Phase High Performance Liquid Chromatographic Separation of the Enantiomers of Terbutaline by Derivatization with 2,3,4,6-Tetra-o-acetyl-β-D-glucopyranosyl Isothiocyanate

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The enantiomers of the bronchodilator terbutaline were separated by reversed-phase high performance liquid chromatography after derivatization with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate(GITC) reagent. The derivatization proceeded quantitatively within 1 h at room temperature. The corresponding diastereomeric thiourea derivatives were well resolved on an ODS column with acetonitrile-acetate buffer as a mobile phase. Elution orders of the diastereomers were confirmed by derivatization of R-(-)-terbutaline and S-(+)-terbutaline which were collected by semi-preparative chiral HPLC using Sumichiral OA-4700 column. The native fluorescence of terbutaline was quenched by derivatization with GITC. The detection limit was 25ng when monitored at UV 278 nm.

Key words: Chiral derivatization, Terbutaline, Enantiomer, GITC, HPLC

INTRODUCTION

Terbutaline is a β_2-adrenoceptor agonist widely used as a racemate in the clinic for the treatment of asthma. The agonistic effect resides in R-(-)-terbutaline and there is no apparent interaction with the less active S-(-)-terbutaline (Jeppson *et al.*, 1984). Interest in the chiral separation of β_2-agonists has grown with the recognition of their enantioselective disposition in humans (Borgström *et al.*, 1989; Walle and Walle, 1990). In addition, the possibility that the inactive (+)-enantiomers of this class of drug may be associated with adverse effects such as bronchial hyperresponsiveness (Kallstrom *et al.*, 1996; Handley, *et al.*, 1998) suggests a 'racemic switch' to pure active enantiomer dosage forms may be beneficial.

The enantiomers can be separated chromatographically using a chiral stationary phase (direct method) or an achiral stationary phase after conversion of the enantiomers into diastereomeric derivatives (indirect method). In the direct method, the enantiomers form transient diastereomeric complexes, which may involve π-π bonds, hydrogen bonds, electrostatic bonds and at least one steric interaction with the chiral stationary phase. The difference in the stabilities of the diastereomeric complexes provides the basis for the separation of the enantiomers. In the indirect method, the enantiomers are first derivatized with an optically pure chiral derivatizing reagent to form a pair of diastereomers. Unlike the enantiomers, the corresponding diastereomers have different physical properties and can be separated chromatographically on an achiral stationary phase. The adventages of the indirect method include the commercial availability of a wide array of chiral derivatizing reagents for the derivatization of various functional groups and high flexibility with respect to chromatographic conditions (Srinivas *et al.*, 1992).

As an indirect separation method, 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate(GITC) which reacts with amino compounds even in aqueous media and has been successfully applied to the chiral separation of DL-amino acids (Kinoshita *et al.*, 1981; Nimura *et al.*, 1984) and β-blocking agents (Sedman and Gal, 1983) was employed. Terbutaline reacted readily with GITC at room temperature and with a small amount of alkali in aqueous media. The corresponding diastereomers were successfully separated by an analytical ODS column.

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This paper describes the reversed-phase HPLC chiral separation of the enantiomers of terbutaline. The optimization of the derivatization procedures and HPLC conditions is investigated.

MATERIALS AND METHODS

Materials and equipment

Triethylamine(TEA) and 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Terbutaline sulfate and trifluoroacetic acid were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). HPLC grade methanol, acetonitrile, *n*-hexane and 1,2-dichloroethane were obtained from J.T.Baker (Phillipsburg, NJ. USA). Water was purified with a Milli-RO 15 Water system (Nihon millipore, Japan) and was filtered through a 0.2 μm membrane filter. All other reagents were of analytical reagent grade. The mobile phase was filtered through a 0.2 μm filter and degassed by sonication under vacuum before using it.

High performance liquid chromatograph was consisted of Shimadzu Model LC-9A pump, SPD-6AV spectrophotometric detector, RF-535 fluorescence detector, SCL-6B system controller and C-R4AD chromatopac (Kyoto, Japan). The separations were carried out on a Inertsil ODS-2 column, 4.6 mm I.D. × 150 mm, with 5 particle size (GL Science Inc, Japan) at ambient temperature. Semi-preparative chiral chromatography was performed with Sumichiral OA-4700 chiral column (8.0mm I.D. × 250 mm, with 5 μm particle size, Osaka, Japan).

Determination of the order of elution

Terbutaline racemate (40 mg) was dissolved in 10 ml of mobile phase. This solution was injected into the semi-preparative chiral HPLC system and resolved into each enantiomer on the Sumichiral OA-4700 column by the *n*-hexane, 1,2-dichloroethane, methanol and trifluoroacetic acid (240 : 140 : 25 : 1, v/v/v/v) as a mobile phase at room temperature and flow rate of 4 ml/min monitoring at UV 278 nm. Fraction containing single enantiomer was collected and evaporated to dryness under nitrogen stream. The direction of rotation (+/-) was determined using a Jasco DIP-1000 digital polarimeter. R-(-)-terbutaline and S-(+)-terbutaline were derivatized with GITC at room temperature for 1 h, followed by injected into the achiral HPLC system (column; Inertsil ODS-2, 5 μm, 4.6 mm I.D. × 150 mm, mobile phase; 36% acetonitrile in 0.025M ammonium acetate buffer (pH=5), flow rate; 1 ml/min) and the elution order of each isomer was confirmed.

Optimization of the mobile phase

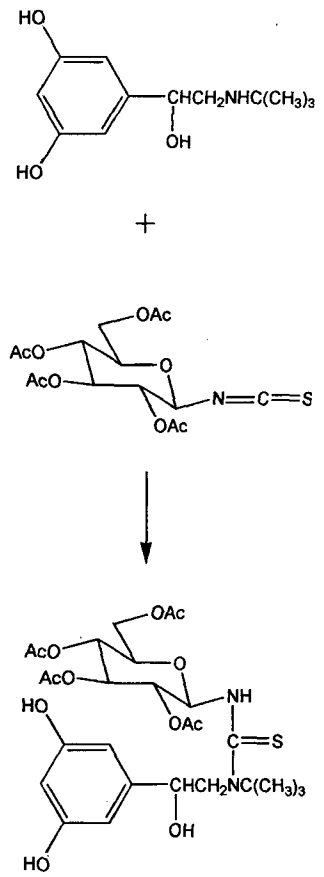

Fig. 1. Proposed reaction of GITC with a secondary amine of a terbutaline to a diastereomeric thiourea

The effects of the changes in the mobile phase pH and ionic strength on the resolution of the diastereomers were investigated. pH of the mobile phase, 36% acetonitrile in 0.025M ammonium buffer, was changed from 3 to 7. The concentration of the ammonium acetate in the mobile phase, 36% acetonitrile in ammonium acetate buffer(pH=5) was changed from 5 to 100 mM.

Optimization of derivatization

Terbutaline enantiomers were dissolved in acetontrile containing a little water (20 μg/ml). To 200 μl of each solution, 50 μl aliquots of various concentrations of GITC solution and triethylamine solution were added and stand for 1 h at room temperature. Following evaporation and dissolution of the residue in mobile phase, the resulting samples were injected into the achiral HPLC system and the peak areas were quantitated.

The effects of time and temperature on the reaction were investigated. To 200 μl of the each solution of enantiomers, 50 μl of GITC solution (3.5 mg/ml of acetonitrile) and triethylamine solution (1.53l μl/ml of

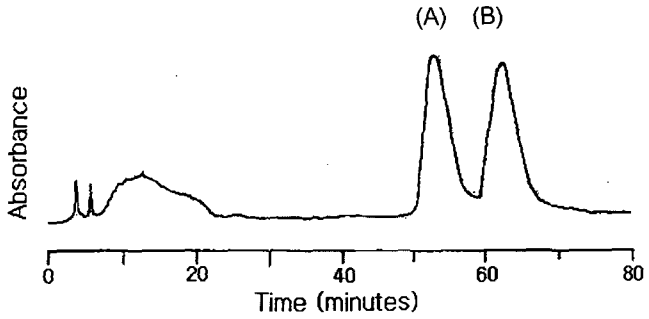

Fig. 2. Chiral semi-preparative HPLC chromatogram of terbutaline racemate. Column; Sumichiral OA-4700 (5 μm, 8×250 mm), mobile phase; *n*-hexane : 1, 2-dichloethane: methanol : trifluoroacetic acid (240 : 140 : 25 : 1,v/v/v/v), flow rate; 4ml/min, detection; UV at 278 nm. Peak (A); S-(+)-terbutaline, peak (B); R-(-)-terbutaline.

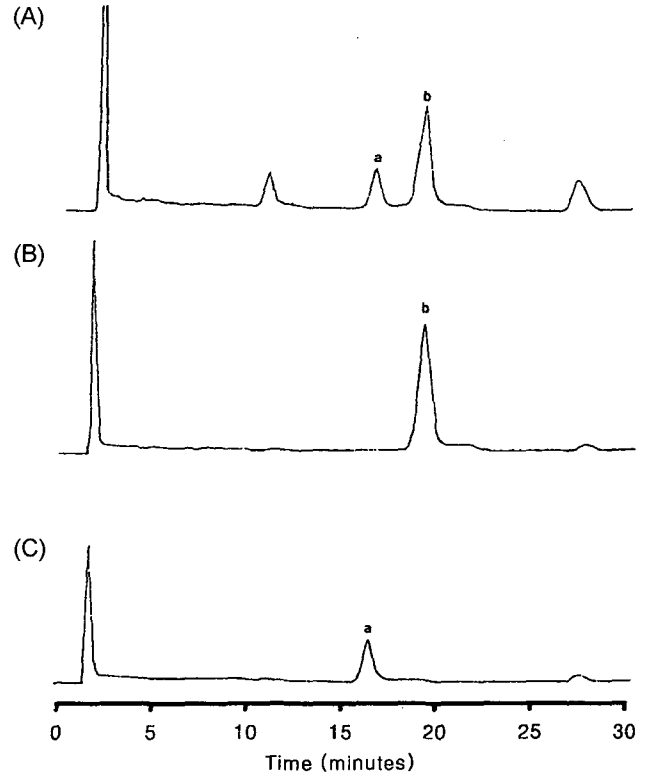

Fig. 3. HPLC chromatograms of (A) terbutaline racemate, (B) R-(-)-terbutaline and (C) S-(+)-terbutaline after fractionation from the chiral semi-preparative HPLC and dervatization with GITC. Column; Inertsil ODS 2 (5 μm, 4.6× 150 mm), mobile phase; 36% acetonitrile in ammonium acetate buffer (pH=5.0), flow rate; 1 ml/min. Peak (a); derivative of R-(-)-terbutaline, peak (b); derivative of S-(+)-terbutaline.

acetonitrile) were added and reacted at room temperature, 45°C, 65 for 15 min, 30 min, 1 h, 2 h, 3 h, or 4 h. They were evaporated to dryness, the residue dissolved in the mobile phase and the resulting

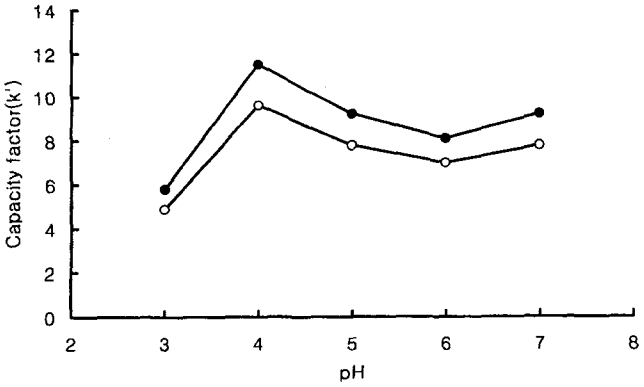

Fig. 4. The effect of pH on the capacity factors of derivatives of terbutaline enantiomers. Mobile phase is 36% acetonitrile in 0.025 M ammonium acetate buffer. ○ ; Derivative of R-(-)-terbutaline, ● ; Derivative of S-(+)-terbutaline.

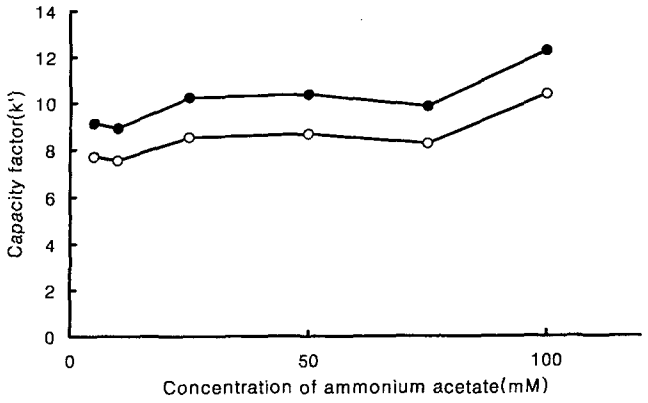

Fig. 5. The effect of ionic strength on the capacity factors of derivatives of terbutaline enantiomers. Mobile phase is 36% acetonitrile in ammonium acetate buffer(pH=5.0). ○ ; Derivative of R-(-)-terbutaline, ● ;Derivative of S-(+)-terbutaline.

samples quantitated by achiral HPLC.

RESULTS AND DISCUSSION

Chiral semi-preparative HPLC of R-(-)- and S-(+)-terbutalinel and determination of elution order of the derivatives

On the chiral semi-preparative HPLC system, S-(+)-terbutalinel was eluted initially (Fig. 2). After fractionation of the eluent containing each enantiomer and derivatization with GITC, the derivative of R-(-)-ter-butalinel was found to be eluted faster than that of S-(+)-terbutaline on the achiral reversed-phase HPLC system (Fig. 3). Racemization was not occurred during derivatization process.

Optimization of the mobile phase

Changes in capacity factors followed a simlar pattern for the two derivatives terbutaline enantiomers(Fig. 4). At pH 4 the capacity factors somewhat were increased and resolution was decreased. At pH 5 best resolution was achieved. Over pH 6 resolution was slightly decreased. Fig. 5 shows that capacity factors increased as the concentration of ammonium acetate increased. In 25 mM of ammonium acetate the resoution was better and the capacity factors were less, relatively. As the concentration of acetontrile increased, the capacity factors and resolution were decreased and the interference peak and the peak of the derivative of R-(+)-terbutaline were well resolved at the lower concentration of acetonitrile.

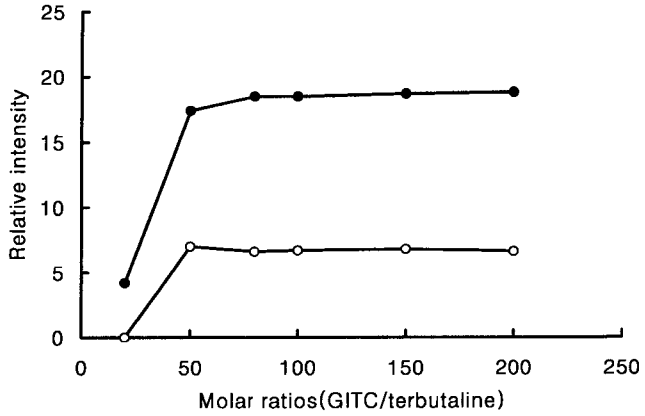

Fig. 6. The effect of GITC concentration on the peak areas of the derivatives of terbutaline enantiomers. ○ ; R-(-)-terbutaline, ● ; S-(+)-terbutaline.

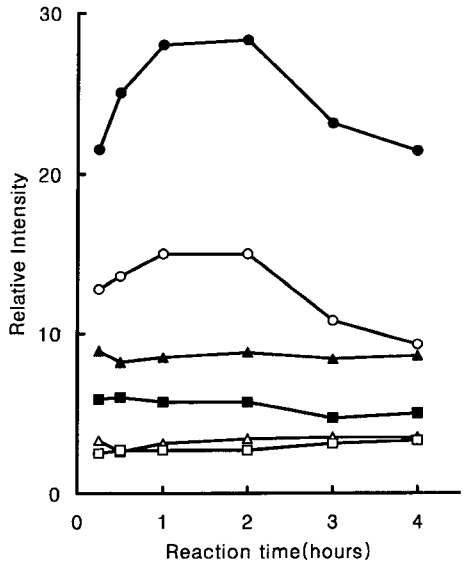

Fig. 7. The effect of reaction temperature and time on the peak areas of the derivatives of terbutaline enantiomers. ○ ; R-(-)-terbutaline at room temperature, ● ; S-(+)-terbutaline at room temperature, △ ; R-(-)-terbutaline at 45°C, ▲ ; S-(+)-terbutaline at 45°C, □ ; R-(-)-terbutaline at 65°C, ■ ; S-(+)-terbutaline at 65°C.

Optimization of derivatization

As shown in Fig. 6, the maximum peak area response for the enantiomers was seen at the 50 times molar excess of GITC. In the final analytical conditions, the GITC concentration was chosen 100 times molar excess in order to provide an adequate excess of reagent. As the reaction temperature was increased from room temperature to 45°C or 65°C, the peak areas of enantiomers were decreased and other impurity peaks supposed to emerge from side reaction were increased. The formation of the derivatives of enantiomers increased with the reaction time up to 1 h at room temperature and reached a plateau and decreased after 2 h (Fig. 7). In conclusion, it was found that the derivatization of terbutaline with GITC was an useful technique for the separation of the enantiomers by reversed phase HPLC. The procedure has several major advantages over previously described techniques: the derivatization is simple and rapid, the chiral reagent (GITC) is commercially available and inexpensive and commercial inexpensive reversed-phase chromatographic column can be used. Good separation of the diasteromeric derivatives was obtained. Because the native fluorescence of terbutaline was quenched, the sensitivity of the method is somewhat poor. The detection limit was 25 ng when monitored at UV 278 nm. However, this method can be applied to the determination of the enantiomeric excess of the terbutaline enantiomer.

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