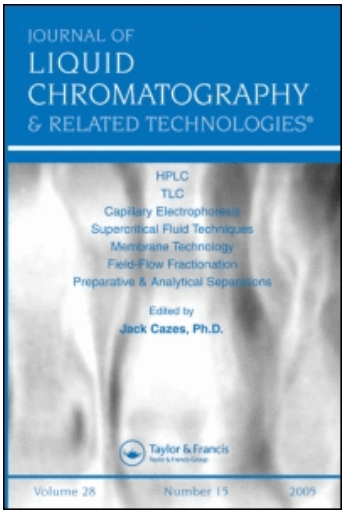

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Determination of the *L*-Enantiomer of Nateglinide in a Bulk Drug Substance by Chiral Reversed-Phase Liquid Chromatography

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ABSTRACT

A simple and practical reversed-phase liquid chromatographic (RP-LC) method is described for the determination of *L*-enantiomer in the bulk drug substance of nateglinide. Enantioseparation was achieved at ambient temperature on a Chiralcel OD-R column (250 × 4.6 mm, 10 μm) using a mobile phase consisting of 0.6 mol/L sodium perchlorate and acetonitrile (48 : 52, v/v, pH 2.0) delivered at a flow rate of 0.2 mL/min. Analytes were monitored at 220 nm. The method is selective and sensitive for this

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purpose with a limit of detection (LOD) and quantitation (LOQ) of 0.3 μg/mL and 0.8 μg/mL for the *L*-enantiomer, and 0.4 μg/mL and 1.0 μg/mL for the *D*-enantiomer, respectively.

Key Words: Nateglinide; Enantiomers; Chiral liquid chromatography.

INTRODUCTION

In the pharmaceutical industry, the separation of enantiomers is growing in interest because the enantiomers of a drug often display quite different pharmacological activity and toxicity profiles. As a result, the U.S. Food and Drug Administration has issued an order to specify the enantiomeric purities of chiral drugs,[1] which increases the demand for direct methods of enantioseparation of chiral drugs.

Chromatographic resolution of enantiomers by liquid chromatography (LC) has gained a great reputation and these methods have become a practically useful approach for determining optical purity and for obtaining individual enantiomers. Among the various chiral columns used, cellulose phenylcarbamate derivatives belong to the most widely used chiral stationary phases for LC enantioseparations.[2–4] They can be used both in normal phase mode (NP-LC)[5] and in reversed-phase mode (RP-LC).[6]

Nateglinide (Fig. 1), *N*-(trans-4-isopropylcyclohexyl-carbonyl)-*D*-phenylalanine, is a highly physiologic mealtime glucose regulator. It can rapidly increase insulin secretion when taken before meals, mimicking early-phase insulin release lost in patients with Type 2 diabetes.[7,8] Only the *D*-enantiomer is approved to be used in clinical treament because it is much more potent than the *L*-enantiomer. But, as the *L*-enantiomer can be introduced during the synthetic process, the bulk drug substance of nateglinide often contains minor amounts of the *L*-enantiomer as an impurity. Therefore, it is necessary to monitor the purity of the bulk drug substance in order to keep level of the *L*-enantiomer under control. In terms of analytical methods for chiral separation of this drug, only two published papers are available,[9,10] which achieved enantioseparation of the two enantiomers using a column packed with amino acid based chiral stationary phase, operated in NP mode. However,

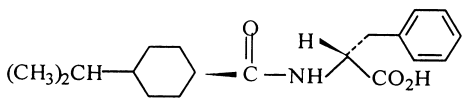

Figure 1. Structure of nateglinide.

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the chromatographic conditions cannot be easily applied owing to the difficulties in obtaining the column and practicability of NP-LC in our laboratory, which prefers a LC method using commercially available chiral column operated in RP mode.

The aim of this work was to develop a chiral RP-LC method for the determination of the *L*-enantiomer in the bulk drug substance of nateglinide which is feasible for the routine quality control of the product.

EXPERIMENTAL

Chemicals and Reagents

Nateglinide reference standard and bulk drug substance, and *L*- enantiomer reference standard were provided by Shenyang Pharmtech Institute of Pharmaceuticals (Shenyang, P.R. China). HPLC-grade acetonitrile was purchased from Fisher Scientific (Springfield, NJ). Distilled water was prepared by using a Milli-Q water-purification system (Millipore, Bedford, MA). All other chemicals were of analytical grade and were used without further purification.

Apparatus

Chromatographic separation was performed with an HP series 1100 chromatographic system equipped with a G1310A Iso Pump, an HP variable UV/VIS detector, a G1328A manual injector with a 20 μL loop (Agilent, CA). Echrom 98 chromatography workstation software was used for instrument control and data acquisition (Elete, P.R. China). Chiralcel OD-R (250 × 4.6 mm, 10 μm), packed with the 3,5-dimethyphenyl-carbamate derivative of amylose, coated on 10 μm silica-gel support, was purchased from Daicel Chemical Industries (Tokyo, Japan). A Shimadzu UV-2201 UV/VIS double-beam spectrophotometer (Shimadzu, Kyoto, Japan) was used for scanning and selecting the detection wavelength.

Chromatographic Conditions

Enantioseparation was achieved at ambient temperature on a Chiralcel OD-R column (250 × 4.6 mm, 10 μm) using a mobile phase consisting of a solution of 0.6 mol/L sodium perchlorate and acetonitrile (48 : 52, v/v, pH 2.0), delivered at a flow rate of 0.2 mL/min. Analytes were monitored at 220 nm. The injected volume was 20 μL.

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Preparation of Test Solution and Control Solution

The test solution (1 mg/mL) was prepared by dissolving approximately 50 mg of the bulk drug substance of nateglinide into a 50 mL volumetric flask and diluting to volume with mobile phase. A control solution was obtained with the *L*-enantiomer reference standard at a concentration of 5 μg/mL in the mobile phase.

RESULTS AND DISCUSSION

Method Development

Cellulose stationary phases (e.g., Chiralcel OD) in HPLC have broad enantiorecognition capabilities for many kinds of chiral compounds, including basic, acidic, and neutral substances.[6] With these stationary phases, the ionization of chiral compounds is the most important parameter to control, in order to achieve good separation of enantiomers. In general, enantiomers can be better separated when they are uncharged. Therefore, it was expected that good enantioseparation of nateglinide (acidic component) would be achieved at a low pH (i.e., 2.0). In terms of the composition of mobile phase, one of the most used phases is a mixture of sodium perchlorate and acetonitrile in the ratio of 60 : 40, with which this study started. As a result, the mixture in the ratio of 60 : 40 (v/v, pH 2.0) did not achieve good enantioseparation of nateglinide. Then, various ratios from 55 : 45 to 45 : 55 were examined to find an optimal phase. It was found that baseline separation was achieved with the ratio of 48 : 52, which was critical for the separation of the two enantiomers. Minor changes of the ratio, such as 47 : 53, 49 : 51 had no significant effect on the resolution between the enantiomers. But, with larger changes, the resolution would deteriorate. A flow rate of 0.2 mL/min was used to achieve baseline separation. Since nateglinide has no significant absorption except for end absorption in the ultraviolet region, the wavelength of 220 nm was adopted for its detection.

Selectivity

A representative chromatogram, for a test solution containing a small amount of *L*-enantiomer, is shown in Fig. 2, which shows that the two enantiomers are well resolved and are displayed as symmetrical peaks with retention times of 41.1 min and 43.3 min, respectively. The resolution between the two enantiomers is larger than 1.5 and column efficiency is more than 7000, expressed as theoretical plate number. The results show that the

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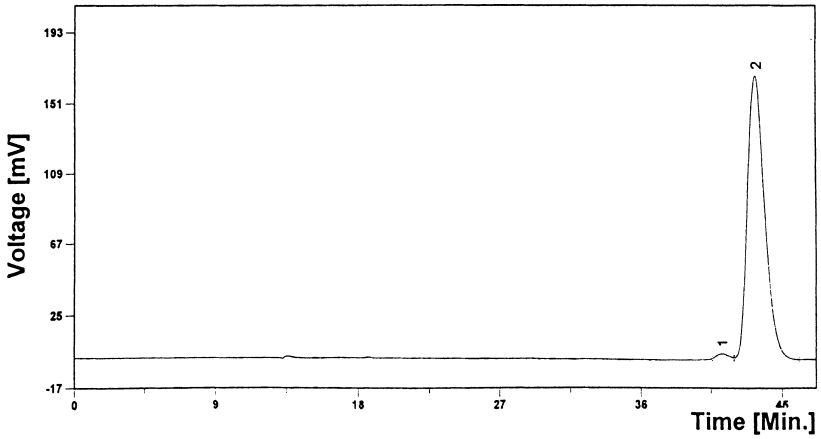

Figure 2. A representative chromatogram of a test solution. Peaks: 1. *L*-enantiomer; 2. *D*-enantiomer.

proposed chromatographic system achieves good resolution and symmetrical peaks for both enantiomers.

Solution Stability

To evaluate the stability of the two enantiomers in the mobile phase, a mixed solution containing both enantiomers (50 μg/mL) was freshly prepared in the mobile phase and then determined at the following time points: 0, 2, 4, 6, 8, and 10 h, at room temperature. The solution was stored with protection from direct sunlight during the test. A solution can be regarded as stable within the given period if no significant changes occur in physical properties (e.g., appearance, clarity, or color of solution), no extra peaks appear, and the relative standard deviations of the peak areas for analytes of interest are less than 2.0%. The results show that the physical properties, peak number, and the peak areas of the solution at a given time period remain unchanged for both enantiomers, indicating that both enantiomers are stable, at least for 10 h.

Limits of Detection and Quantitation

The limit of detection (LOD) was established by determining the concentration of a dilute solution of each enantiomer that gave a signal-to-noise ratio of 3. The limit of quantitation (LOQ) is defined as the lowest

concentration that can be determined with acceptable accuracy and precision, which can be established at a signal-to-noise ratio of 10. The LOQ of each enantiomer was experimentally verified by five injections of each enantiomer at its LOQ concentration. The LOD and LOQ values were 0.3 μg/mL and 0.8 μg/mL for the *L*-enantiomer, and 0.4 μg/mL and 1.0 μg/mL for the *D*-enantiomer, respectively.

Determination of *L*-Enantiomer in the Drug Substance

The developed LC method was applied to the determination of the *L*-enantiomer as a type of impurity in several batches of the bulk drug substance of nateglinide. Both the test solution and the control solution were analyzed under the proposed conditions. The mass fraction of the *L*-enantiomer for three batches of drug substance was below the LOQ.

CONCLUSION

The developed method offers simplicity and sufficient sensitivity for the determination of *L*-enantiomer of nateglinide in the bulk drug substance, which can be applied in laboratories for the routine quality control of nateglinide.

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