ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
30 (2002) 823–836

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Direct chiral separation of troglitazone stereoisomers using reversed-phase high-performance liquid chromatography

Nobuyuki Suzuki [a,*], Akira Takemura [a], Akifumi Miyamoto [a], Takao Yoshioka [b], Shinya Tsutsumi [c], Takao Kawasaki [a]

[a] *Product Development Laboratories, Sankyo Co., Ltd, 2-58, Hiromachi 1-chome, Shinagawa-ku, Tokyo 140-8710, Japan*
[b] *Medicinal Chemistry Research Laboratories, Sankyo Co., Ltd, 2-58, Hiromachi 1-chome, Shinagawa-ku, Tokyo 140-8710, Japan*
[c] *Biomedical Research Laboratories, Sankyo Co., Ltd, 2-58, Hiromachi 1-chome, Shinagawa-ku, Tokyo 140-8710, Japan*

Received 16 March 2002; received in revised form 7 June 2002; accepted 12 June 2002

Abstract

A simple HPLC method for the direct chiral separation of troglitazone stereoisomers was developed. The separation was performed on a reversed-phase cellulose-derivertized chiral column (Chiralcel OJ-R) using a mobile phase consisting of methanol–acetic acid (1000:1, v/v) at a flow rate of 0.5 ml/min. The peak areas of stereoisomers separated from 0.13 to 0.75 mg/ml of troglitazone had good linearity, with correlation coefficients >0.999 in the reversed-phase mode. The repeatability of the ratios of stereoisomers isolated from 0.5 mg/ml of troglitazone had a relative standard deviation of 0.1–0.2%. The relative sensitivities of the four isomers at UV 285 nm were similar, as each response factor was within the range of 0.99–1.01. Troglitazone racemized at the chiral center of the thiazolidine ring in methanol solution, but was found to be stable for 24 h in methanol–acetic acid (1000:1, v/v). This method was applied to the stereoisomeric analysis of troglitazone in pharmaceutical formulations and used to evaluate the constancy of the stereoisomer ratio in the manufacturing process and stability testing.

Keywords: Troglitazone; Anti-diabetic drug; Chiral separation; Reversed-phase LC; Chiral column

1. Introduction

Separation of enantiomers has become very important in analytical chemistry, especially in the pharmaceutical and biological fields, because some stereoisomers of racemic drugs have quite different pharmacokinetic properties and different pharmacological or toxicological effects [1–3]. That is one of the most important reasons why the regulatory authorities demand more stringent investigations for evaluating the safety and effectiveness of drugs containing asymmetric centers.

Troglitazone is a novel oral anti-diabetic drug [4,5] that improves insulin sensitivity and responsiveness. Troglitazone also lowers hepatic glucose production. This compound is not only effective in insulin-dependent diabetes mellitus patients, but also in non-insulin-dependent diabetes mellitus patients [6,7]. Some HPLC methods have been

* Corresponding author. Tel.: +81-3-3492-3131; fax: +81-3-5436-8571
E-mail address: nobuyu@shina.sankyo.co.jp (N. Suzuki).

0731-7085/02/$ - see front matter
PII: S0731-7085(02)00391-6

reported and validated for the analysis of troglitazone in pharmaceutical and biological samples [8–11].

Troglitazone has two asymmetric carbons, one at the 2-position of the chroman ring and one at the 5-position of the thiazolidine ring in its molecule, and is produced as a mixture of equal amounts of four optical isomers. When a solid state of racemic compounds having diastereomers, such as troglitazone, is elucidated, accurate estimation of the optical isomer ratio is absolutely essential.

In this study, we developed and validated a rapid and efficient direct HPLC method with a chiral column for the determination of troglitazone stereoisomers. Furthermore, we applied this chiral separation method to the evaluation of the stereoisomers of troglitazone in pharmaceutical formulations and their in-process materials.

2. Materials and methods

2.1. Sample and reagents

Troglitazone bulk drug substance (Lot No. NR312) used in this study was synthesized by the Process Development Laboratories, Sankyo Co., Ltd. The troglitazone drug products, 100-mg tablet (Lot No. CH002) and 200-mg tablet (Lot No. CH043), and their in-process materials were obtained from the Product Development Laboratories, Sankyo Co., Ltd. All other reagents and solvents were commercially available and of analytical reagent grade.

2.2. Equipment

Chiralcel OJ (4.6 mm i.d. × 250 mm, particle size: 10 μm), Chiralcel OJ-R (4.6 mm i.d. × 150 mm, particle size: 5 μm), Chiral-AGP (4.6 mm i.d. × 250 mm, particle size: 5 μm) and Chiralcel OA (4.6 mm i.d. × 250 mm, particle size: 10 μm) were purchased from Daicel Chemical Industries.

An HP1090 high-performance liquid chromatography system (Hewlett-Packard), and an HP1100 UV detector (Hewlett-Packard) were used.

Water for preparing buffers and mobile phases was used after purification with a MilliQ-SP TOC system (Millipore).

2.3. Synthesis and isolation of four stereoisomers of troglitazone

The 2R–5R (ROY-1585), 2R–5S (ROY-1586), 2S–5R (ROY-1587), and 2S–5S (ROY-1588) stereoisomers are shown in Fig. 1 with the compound names and chemical structures. The 2R–5R/2R–5S form of troglitazone (2R) and the 2S–5R/2S–5S form of troglitazone (2S) were synthesized from the optically active 2-methyl-5-oxotetrahydro-2-furoic acid. Absolute configuration at the 2-position of the chroman ring was established in a study of vitamin E [12]. The 2R-troglitazone (m.p. 165.3 °C) and 2S-troglitazone (m.p. 165.8 °C) were obtained from configured 2-chroman-2-methanol using the same method as the synthesis of troglitazone drug substance [4].

For the isolation of each stereoisomer, 2R-troglitazone was resolved into 2R–5R and 2R–5S and 2S-troglitazone was resolved into 2S–5R and 2S–5S by HPLC using a Chiralcel OA column. From 3.2 g of 2R-troglitazone, 180 mg of the front component (>99% e.e., m.p. 139.7 °C, $[\alpha]_D^{20} = -97.3°$) and 145 mg of the rear component (97.8% e.e., m.p. 92.4 °C, $[\alpha]_D^{20} = +110.0°$) were obtained. And from 3.5 g of 2S-troglitazone, 276 mg of the front component (>99% e.e., m.p. 87.2 °C, $[\alpha]_D^{20} = -108.4°$) and 141 mg of the rear component (97.2% e.e., m.p. 141.7 °C, $[\alpha]_D^{20} = +99.0°$) were obtained.

The RR/SS and the RS/SR forms, diastereomers, were separated from the troglitazone drug substance by recrystallization. Troglitazone (22 g) was dissolved in acetone, concentrated and dissolved in HCl–ethanol, kept at room temperature. Crude crystals and filtrate were separated and the recrystallization process described above was repeated many times. Purified rectangular crystals (~5 g in total, m.p. 195.5 °C) were obtained from the crude crystals, and were identified as RS/SR form by X-ray analysis [13]. Rhombic crystals (~2 g in total, m.p. 121.6 °C) were obtained from the filtrate by excluding the rectangular crystals, and were identified as the RR/SS form [14].

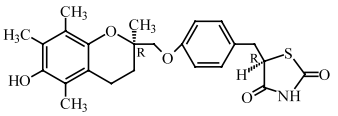
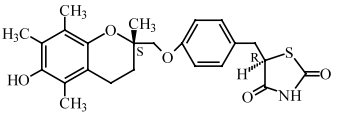
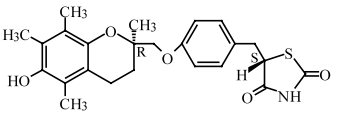
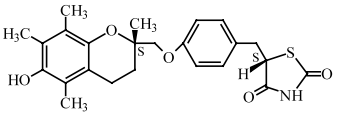
Stereoisomer	Compound name	Chemical structure
2R-5R	ROY-1585	
2S-5R	ROY-1587	
2R-5S	ROY-1586	
2S-5S	ROY-1588	

Fig. 1. Compound names and chemical structures of troglitazone stereoisomers.

2.4. Sample preparation

For the preparation of a sample solution in reversed-phase mode with Chiralcel OJ-R, 10 mg of troglitazone was transferred into a 20-ml volumetric flask, and was dissolved and diluted to volume with methanol–acetic acid (1000:1, v/v). This solution (0.5 mg troglitazone/ml) was used as the sample solution for the reversed-phase HPLC method. In the normal-phase mode with Chiralcel OJ, 50 mg of troglitazone was dissolved in dehydrated ethanol to make 20 ml for the preparation of the sample solution (2.5 mg troglitazone/ml). For the other normal-phase HPLC methods, the sample solution was prepared by dissolving with the respective mobile phases in the same way.

For the analysis of troglitazone drug product, the following preparation was needed. One tablet containing either dose of troglitazone was weighed and finely powdered. A portion of the powder equivalent to 50 mg of troglitazone was weighed precisely ($\sim$130 mg), transferred to a 100-ml volumetric flask, and 100 ml of methanol–acetic acid (1000:1, v/v) was added. The flask was placed in an ultrasonic water bath for 10 min and an aliquot of about 30 ml of suspended solution was centrifuged at 3000 rpm ($\sim 2200 \times g$) for 10 min. Five-microliter aliquots of the supernatant were injected for HPLC analysis.

2.5. HPLC conditions

Separation of troglitazone stereoisomers was carried out using the following HPLC conditions. Reversed-phase chromatography on the Chiralcel OJ-R was performed using a mobile phase composed of methanol–acetic acid (1000:1, v/v) at a flow rate of 0.5 ml/min. The column temperature was controlled at 25 °C with an injection volume of 5 μl, and detection was achieved at UV 285 nm.

In the normal-phase, chromatography on the Chiralcel OJ column was performed using a mobile phase composed of *n*-hexane–dehydrated

ethanol–methanol–trifluoroacetic acid (120:50:30:1, v/v/v/v) at a flow rate of 0.5 ml/min. The column temperature was controlled at 40 °C with an injection volume of 5 μl, and detection was achieved at UV 285 nm.

In addition, a Chiral-AGP column containing α-1 acid glycoprotein was used with a mobile phase composed of 10 mmol/l phosphate buffer (pH 7.0)–methanol (17:3, v/v). And a Chiralcel OA column containing chiral triacetate derivative of cellulose was also used with a mobile phase composed of *n*-hexane–ethanol–phosphoric acid (700:300:1, v/v/v). The conditions of the other normal-phase HPLC methods were the same as those described above.

3. Results and discussion

3.1. Assignment of the absolute configuration of each stereoisomer

For the assignment of the absolute configuration of each of the four isolated stereoisomers, troglitazone was analyzed by HPLC with Chiralcel OJ. The four troglitazone stereoisomers were eluted individually and their peaks were assigned as A, B, C and D in turn as they appeared on the chromatogram. 2R-Troglitazone having a known absolute configuration at the 2-position of the chroman ring gave peaks A and C, and 2S-troglitazone gave peaks B and D. The RS/SR form of troglitazone, including the 2R–5S and 2S–5R stereoisomers gave peaks B and C, and the RR/SS form including the 2R–5R and the 2S–5S stereoisomer gave peaks A and D. Table 1 shows the correlation between the stereoisomer components of the troglitazone substance and the peaks on the HPLC chromatograms. Therefore, each of the peaks A, B, C, and D, corresponds to 2R–5R, 2S–5R, 2R–5S and 2S–5S stereoisomers of troglitazone, respectively. This observation concurs with the result obtained by CD spectrometry as shown in Fig. 2. Since 2R–5R and 2S–5S are enantiomers, the CD spectra are symmetric to each other, and 2S–5R and 2R–5S were the same. On the other hand, the spectrum of 2R–5R (2S–5S) is not very similar to that of 2S–5R (2R–5S), because they are diastereomers.

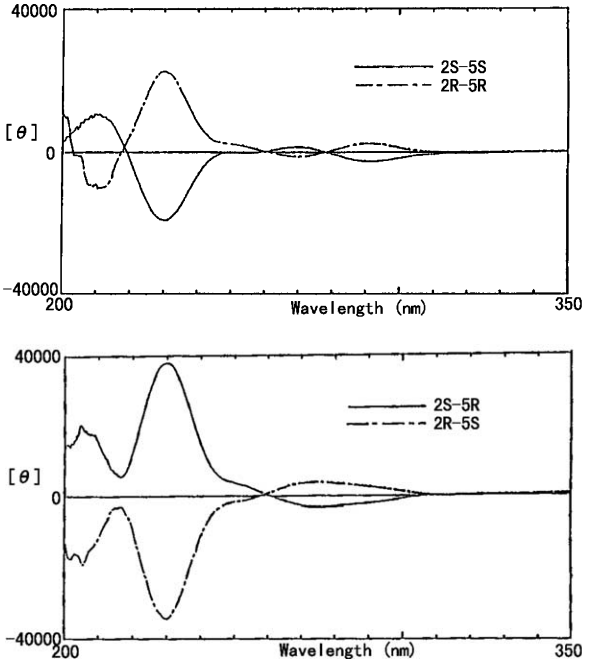


Fig. 2. CD spectra of the four troglitazone stereoisomers.

Table 1
Correlation of the stereoisomer components of the troglitazone substance to peaks on HPLC chromatograms

Compound	Stereoisomer components	Peaks			
Troglitazone	2R–5R, 2R–5S, 2S–5R, 2S–5S	A	B	C	D
2R-Troglitazone	2R–5R, 2R–5S	A		C	
2S-Troglitazone	2S–5R, 2S–5S		B		D
RS/SR form	2R–5S, 2S–5R		B	C	
RR/SS form	2R–5R, 2S–5S	A		D	

3.2. Development of chiral separation

Some polysaccharide or some protein-bound materials are widely used for the chiral stationary phase on the commercially available chiral column. α-1 Acid glycoprotein (α-1 AGP) and cellulose derivatives are both well known as good chiral selectors. Besides, it is possible for cellulose derivatives to induce various types of functions for changing the interaction pattern to the analyte. The chiral carbon at the 2-position of the chroman ring of troglitazone is close to the aromatic ring in the chroman ring regarding the chemical structure. It was predicted that the chiral recognition would be varied according to the presence or absence of the aromatic ring, which causes π–π interaction, in the stationary phase.

In order to select the chiral selector, we compared the separation performance of troglitazone stereoisomers with Chiral-AGP, Chiralcel OA and Chiralcel OJ. The structures of the stationary phases of these chiral columns are depicted in Fig. 3. Fig. 4 shows the optimized chromatograms of troglitazone using Chiral-AGP, Chiralcel OA, Chiralcel OJ and Chiralcel OJ-R. 5R-Troglitazone and 5S-troglitazone could be separated with Chiralcel OA, and all four stereoisomers could be separated individually with Chiralcel OJ and Chiralcel OJ-R. The result indicates that the helical structure or some other structural element of the cellulose molecule has a better chiral resolution on the 5-position of the thiazolidine ring than the α-1 AGP. And it was revealed that the chiral recognition at the 2-position of troglitazone was improved after derivertizing the aromatic ring in the stationary phase, tris(4-methylbenzoate) cellulose in Chiralcel OJ and Chiralcel OJ-R, comparing to the non-resolution with Chiralcel OA. In this way, we selected the tris(4-methylbenzoate) cellulose-immobilized column for the chiral separation of troglitazone in this study.

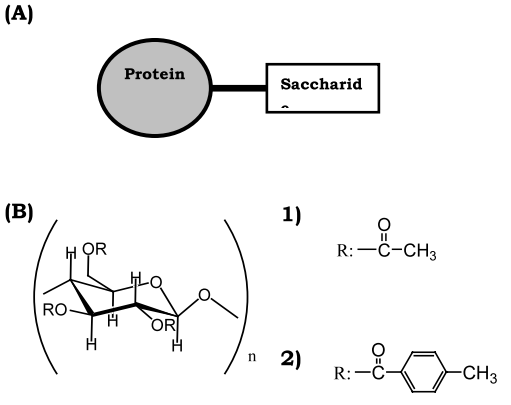


Fig. 3. The structure of the chiral selector immobilized on the stationary phase of the chiral column. (A) Chiral-AGP; (B) (1) Chiralcel OA, (2) Chiralcel OJ and OJ-R.

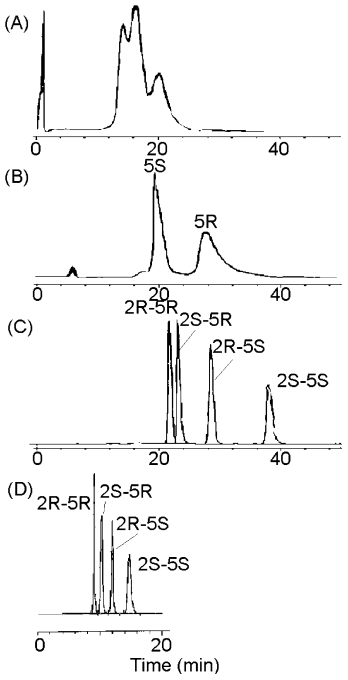


Fig. 4. Optimized HPLC chromatograms of troglitazone with several chiral columns. (A) Column: Chiral-AGP (4.6 mm i.d. × 250 mm), mobile phase: 10 mmol/l phosphate buffer (pH 7.0)–methanol (17:3, v/v), (B) Column: Chiralcel OA (4.6 mm i.d. × 250 mm), mobile phase: *n*-hexane–ethanol–phosphoric acid (700:300:1, v/v/v), (C) Column: Chiralcel OJ (4.6 mm i.d. × 250 mm), mobile phase: *n*-hexane–dehydrated ethanol–methanol–trifluoroacetic acid (120:50:30:1, v/v/v/v) and (D) Column: Chiralcel OJ-R (4.6 mm i.d. × 150 mm), mobile phase: methanol–acetic acid (1000:1, v/v).

Tris(4-methylbenzoate) cellulose is the chiral selector in Chiralcel OJ and Chiralcel OJ-R, which is efficient in the direct resolution of many chiral pharmaceutical compounds [15–18]. HPLC chromatograms in normal-phase mode and reversed-phase mode, using Chiralcel OJ and Chiralcel OJ-

R are shown in Fig. 4(C) and (D), respectively. The four stereoisomers were eluted in the same order for the two methods. This result indicates that each troglitazone enantiomer has an individual affinity with the chiral selector in the stationary phase, and the order of elution had little effect on the polarity of the mobile phase. However, the normal-phase mode needs a long time for analysis (50 min) and is not safe or hygienic because large quantities of non-polar solvents are needed. Furthermore, better separation was obtained in the reversed-phase mode. Thus, a reversed-phase HPLC method is more suitable than a normal-phase HPLC method for application in pharmaceutical and biological sample analyses. We, therefore, selected the reversed-phase mode as the HPLC method and optimized the conditions using Chiralcel OJ-R as described in detail below.

In order to optimize the resolution of each troglitazone enantiomer, the following parameters were investigated: the pH of the mobile phase, the kind of organic solvent and its concentration in the mobile phase, the kind of acid and its concentration in the mobile phase, and the column temperature.

Troglitazone is an acidic compound whose pK_a of the imino group in the thiazolidine ring is 6.1 by the titration method [19]. The retention of these stereoisomers is presumed to be influenced by the pH of the mobile phase at and around the pKa, due to the dissociation of the imino proton. Resolution behavior of the stereoisomers was examined by employing different pHs, 3.0, 4.0, 5.0, 6.0, and 7.0, using acetate and phosphate buffers. As shown in Fig. 5, the isomers eluted rapidly with bad resolution at pH 6.0 and above. At pH 5.0 and below, all four isomers were retained and separated completely. And the retention times and resolutions of the isomers were not changed by using buffer of pH 3.0 or 4.0 or water as the aqueous element of the mobile phase. These results could be caused by depression of the dissociation of the imino residue in the thiazolidine ring.

The effect of organic solvent concentration on separation of stereoisomers was investigated by employing different methanol and water ratios of

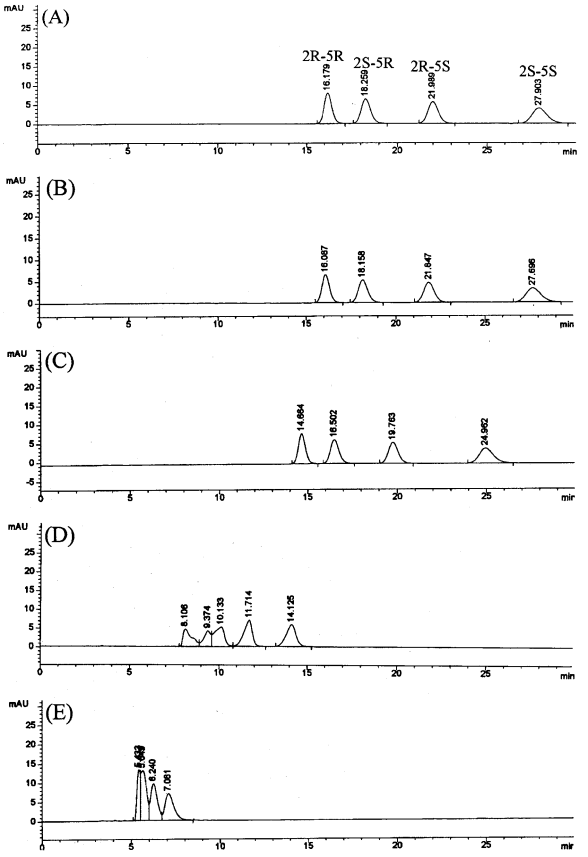


Fig. 5. HPLC chromatograms of troglitazone in various pH eluents. (A) Eluent: methanol–water–acetic acid (950:50:1, v/v/v), (B) eluent: methanol–10 mmol/l acetate buffer pH 4.0 (19:1, v/v), (C) eluent: methanol–10 mmol/l acetate buffer pH 5.0 (19:1, v/v), (D) eluent: methanol–10 mM phosphate buffer pH 6.0 (19:1, v/v), and (E) eluent: methanol–10 mmol/l phosphate buffer pH 7.0 (19:1, v/v).

18:2, 19:1 and 20:0 under acidic conditions with acetic acid. As shown in Fig. 6, the isomers were eluted faster with decreasing ratio of water. The four stereoisomers could be separated completely using methanol–acetic acid (1000:1, v/v) as the mobile phase, and the apparent pH value of the solution was 4.3.

Fig. 7 shows the HPLC chromatograms of troglitazone that were obtained under column temperatures of 40, 25 and 10 °C. Retention times of all stereoisomers were decreased at 40 °C, and resolution between 2R–5R and 2S–5R became incomplete. At 10 °C, the eluted peaks were broad

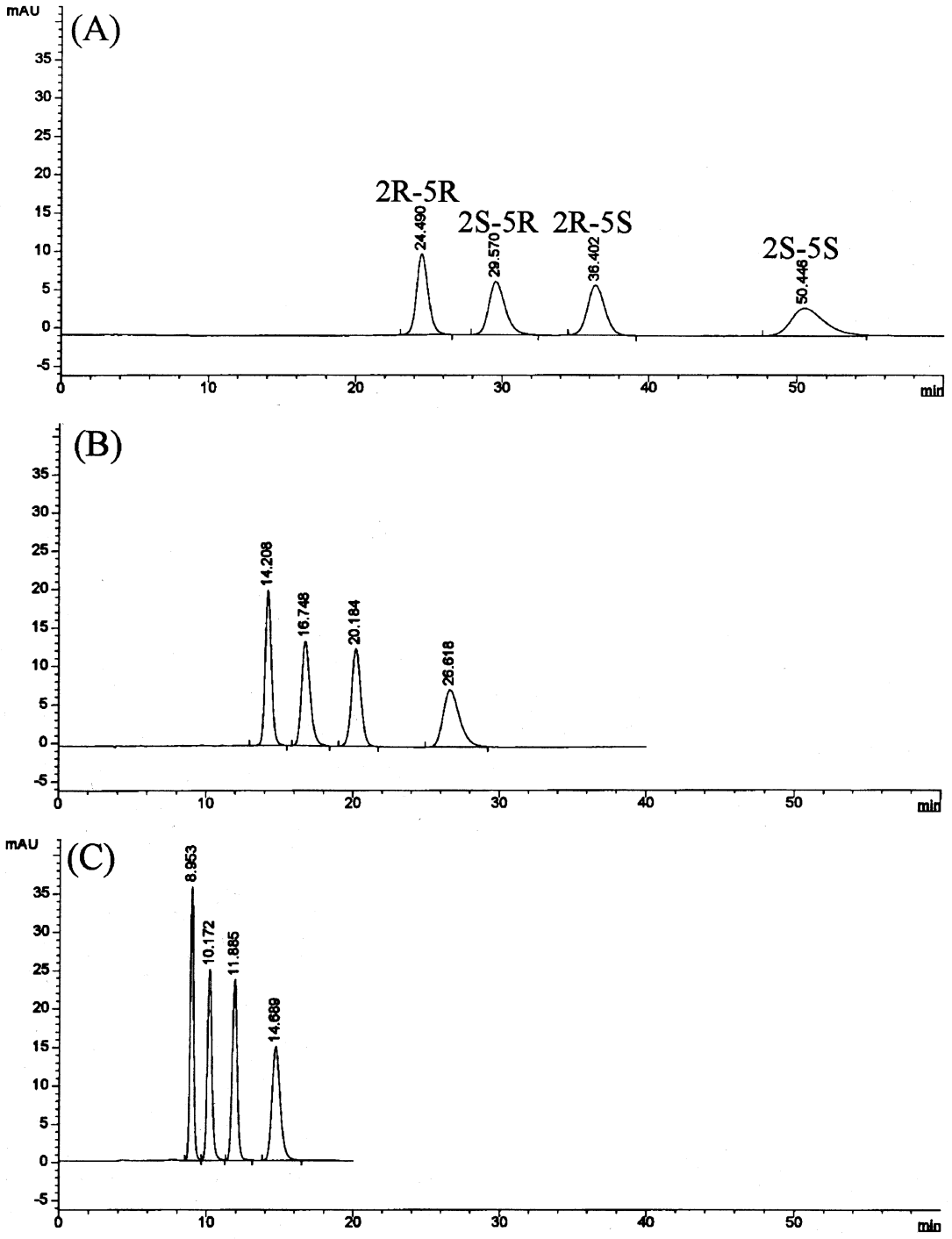


Fig. 6. HPLC chromatograms of troglitazone in eluents with various concentrations of methanol. (A) Eluent: methanol–water–acetic acid (900:100:1, v/v/v), (B) eluent: methanol–water–acetic acid (950:50:1, v/v/v), and (C) eluent: methanol–acetic acid (1000:1, v/v).

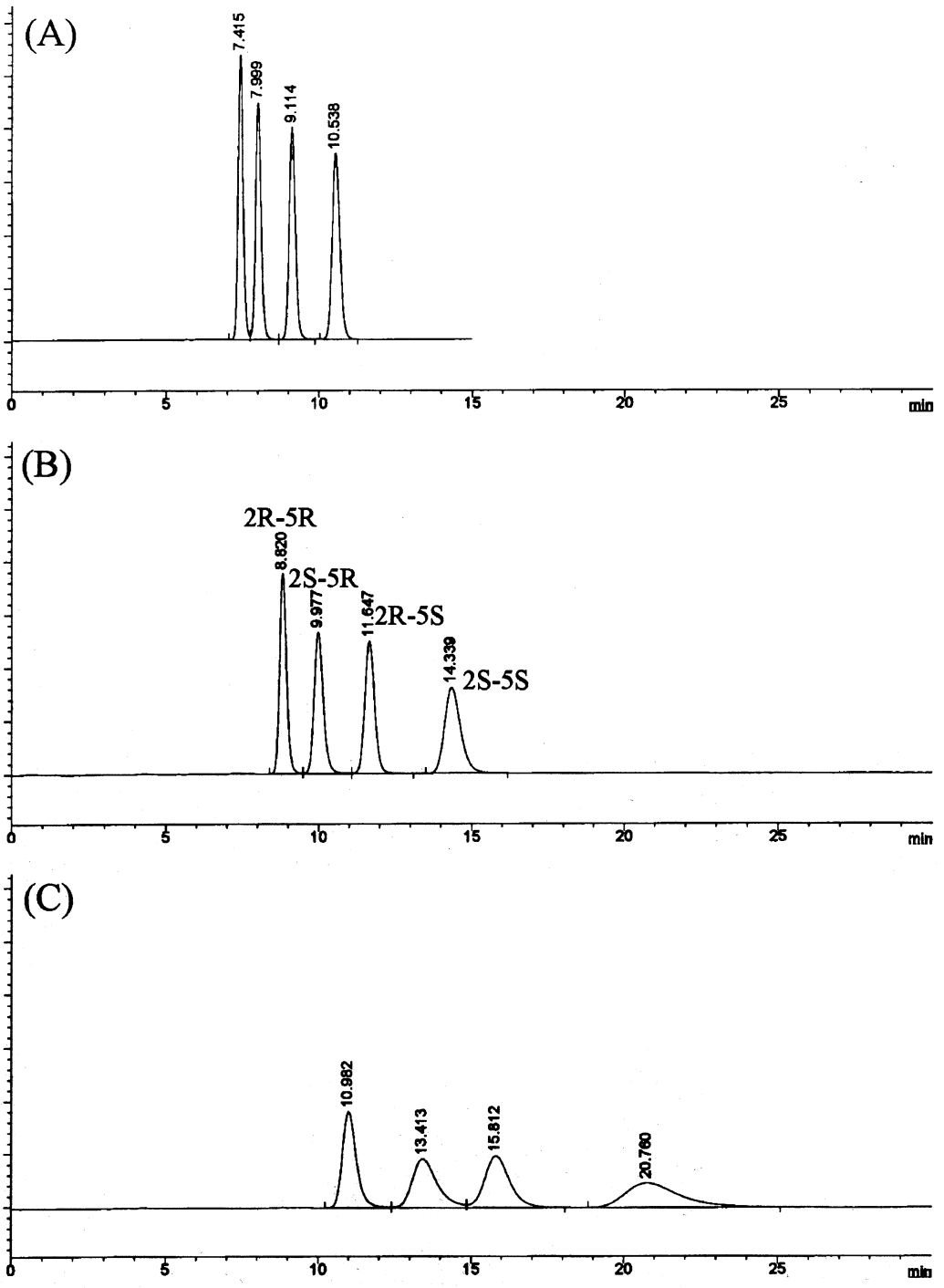


Fig. 7. HPLC chromatograms of troglitazone at various column temperatures at (A) 40 °C, (B) 25 °C, and (C) 10 °C.

and resolution between 2S–5R and 2R–5S was poor. The column temperature adopted was 25 °C because complete separation between all four peaks was obtained at this temperature.

Methanol, ethanol, isopropanol and acetonitrile can be used as a mobile phase for the column. Separation behavior was examined using methanol–acetic acid (1000:1, v/v), ethanol–acetic acid (1000:1, v/v), isopropanol–acetic acid (1000:1, v/v), and acetonitrile–acetic acid (1000:1, v/v). As shown in Fig. 8, complete separation of the four isomers could not be achieved with ethanol, isopropanol or acetonitrile. Methanol was chosen as the organic solvent in the mobile phase because complete separation was obtained with methanol as shown in Fig. 8(A).

Separation characteristics may be changed by the kind of acid used in the mobile phase. Phosphoric acid, perchloric acid and acetic acid were each mixed individually with methanol (1000:1 v/v methanol–acid) and used as the mobile phase. The influence of these three kinds of acids on the chromatographic separation was compared with each other; however, no particular difference was observed among these chromatograms. The separation behavior was also examined by changes in concentration. No change was observed in the chromatograms that were obtained with 500:1, 1000:1, or 2000:1 v/v methanol–acetic acid. Finally 1000:1 v/v methanol–acetic acid was chosen as the mobile phase because it seems that dissociation of troglitazone is completely depressed when using this, so it is stable for separation.

3.3. Analytical validation

In order to validate the HPLC method for use as the routine method, linearity of calibration curve, precision, accuracy and ruggedness were investigated.

For the purpose of determination of the relationship between the concentrations and peak areas obtained by this HPLC method, a series of standard solutions in the range of 25–150% of the nominal sample concentration (0.5 mg/ml) were prepared and analyzed. The peak areas and concentrations of each troglitazone stereoisomer were plotted as ordinate (Y) and abscissa (X), respectively. On substituting the experimental values for quantities X and Y into the exponential regression equation $Y = aX^n$ (where 'a' is the slope), n was found to be 1.001 (2S–5R) to 1.014 (2S–5S). The results indicated the calibration curves were straight lines with good fit. Calibration lines for the four stereoisomers substituted into the linear regression equation $Y = aX + b$ (where 'a' is the slope and 'b' is the Y intercept) were linear ($R > 0.9999$) through the origin as shown in Fig. 9. The peak areas of the four stereoisomers of troglitazone were proportional to the respective concentrations: $Y = 602.62X - 2.719$ (2R–5R), $Y = 561.91X + 1.355$ (2S–5R), $Y = 568.04X + 1.343$ (2R–5S), and $Y = 600.41X - 5.165$ (2S–5S).

Troglitazone exhibits two UV absorption maxima at 225 and 285 nm in acidic solution. To avoid interference from uncertain impurities, 285 nm was chosen for the detection wavelength. Since only a few chemical impurities absorb short wavelength light, a longer wavelength is more likely to have higher selectivity.

Absorbance of a 0.1% solution (0.5 μg/ml) of a nominal sample solution was measured at 285 nm, and concentrations at a signal to noise ratio of 3:1 were calculated for inspection of the limit of detection (LOD). As shown by the LOD of the four stereoisomers in Table 2, the detection sensitivity of this reversed-phase method is higher than that of the normal-phase HPLC method.

In order to examine injection repeatability, the sample solution prepared according to the method of preparation of the sample solution (0.5 mg/ml) was injected 6 times, and the peak areas of each stereoisomer were measured. In order to examine the precision of the analytical procedure, six different sample solutions prepared from the same bulk lot were also analyzed, and method repeatability was examined. The results of the precision of the HPLC injection and the method repeatability are summarized in Table 3. Good injection repeatability was obtained because the relative standard deviation of the peak areas of each stereoisomer was 0.18–0.32%. The relative standard deviation of the stereoisomer ratio was 0.10–0.27%, giving a good result. Method repeatability was also evaluated, the relative standard

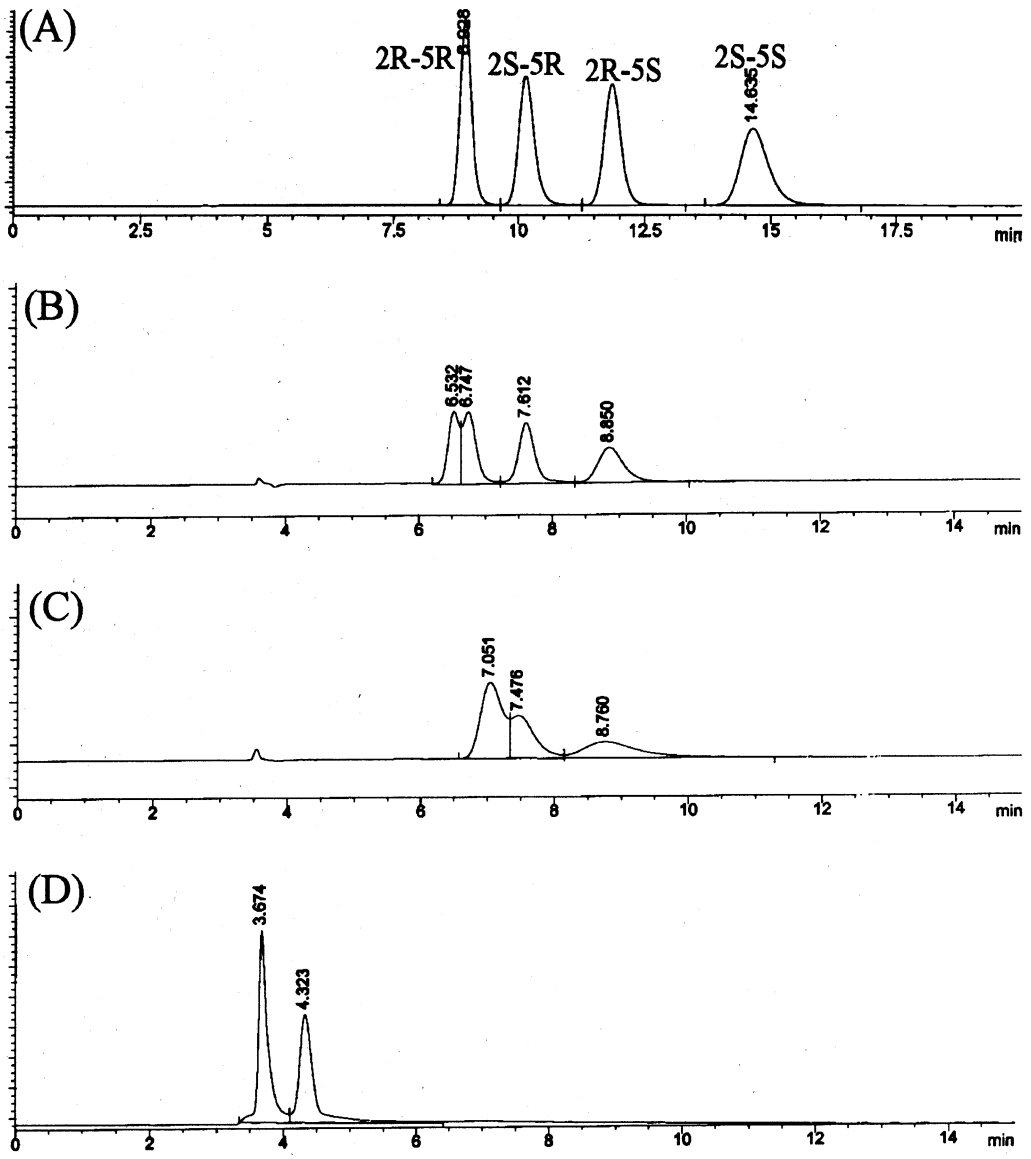


Fig. 8. HPLC chromatograms of troglitazone in eluents with various organic solvents. (A) Eluent: methanol–acetic acid (1000:1, v/v), (B) eluent: ethanol–acetic acid (1000:1, v/v), (C) eluent: isopropanol–acetic acid (1000:1, v/v), and (D) eluent: acetonitrile–acetic acid (1000:1, v/v).

deviation of stereoisomer ratio was 0.09–0.21%. Thus, it was confirmed that this HPLC method has good precision.

Solutions of each stereoisomer (0.125 mg/ml), one fourth of the nominal concentration, were prepared and measured by this HPLC method for investigation of accuracy. Table 4 shows the ratios of the relative sensitivity of each stereoisomer against the 2R–5R isomer of troglitazone. Since the ratios of all the four stereoisomers are almost the same (0.99–1.01), reference standards of the isomers and correction are not needed for the assay; the stereoisomer ratio can be determined accurately by evaluating the peak area percentage.

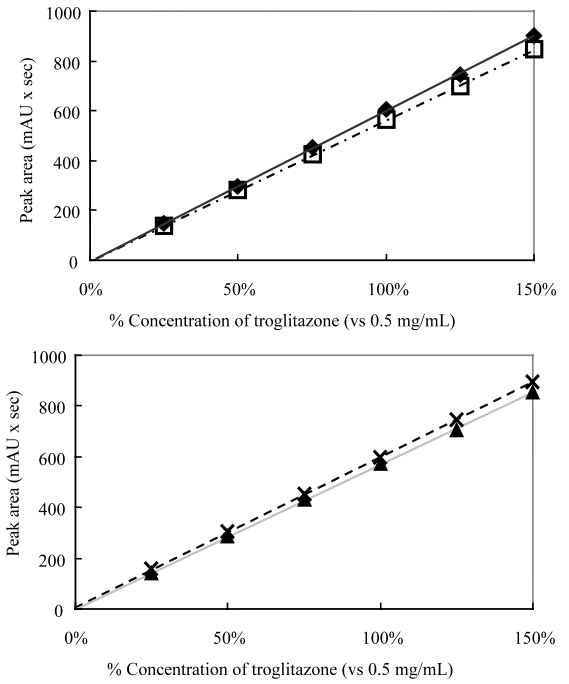


Fig. 9. Calibration curves of troglitazone stereoisomers in the range of 0.125–0.75 mg/ml of troglitazone. 2R–5R (◆), 2S–5R (□), 2R–5S (▲), (×) 2S–5S.

Table 2
Comparison of reversed-phase HPLC method with normal-phase HPLC method for LOD of troglitazone stereoisomers ($S/N = 3$)

Stereoisomer	Reversed-phase (ng/injection)	Normal-phase (ng/injection)
2R–5R	0.8	4.7
2S–5R	1.0	5.3
2R–5S	1.0	5.9
2S–5S	1.7	4.0

Table 4
Relative response factors of troglitazone stereoisomers against 2R–5R troglitazone

Stereoisomer	Relative response factor
2R–5R	1.00
2S–5R	0.99
2R–5S	1.01
2S–5S	1.00

For investigating the stability of the sample solution, each isomer was dissolved in methanol or methanol–acetic acid (1000:1, v/v) to 0.125 mg/ml solutions and analyzed, and the change in the chromatograms was observed. No other isomer was found in the mixture of methanol–acetic acid (1000:1, v/v) even after 24 h, however, in methanol, a change in the absolute configuration at the 5-position of the thiazolidine ring was observed in the chromatogram. From these results, it may be concluded that racemization at the 5-position of the thiazolidine ring occurred using methanol as the mobile phase, and this racemization was depressed by adding acid. The results of racemization of each isomer with time are shown in Fig. 10. No degradation peak was observed during the period of the experiments.

3.4. Application to pharmaceutical formulations and manufacturing process materials

In order to demonstrate the applicability of the proposed method to the separation of troglitazone stereoisomers, the method was applied to the analysis of the drug product. Two hundred and fifty milligrams of troglitazone was dissolved in 100 ml of methanol–acetic acid (1000:1, v/v) for

Table 3
Injection repeatability and method repeatability of troglitazone stereoisomers

Stereoisomer	Injection repeatability ($n = 6$)		Method repeatability ($n = 6$)	
	Average	RSD (%)	Average	RSD (%)
2R–5R	25.9	0.17	25.9	0.12
2S–5R	24.1	0.11	24.1	0.09
2R–5S	24.3	0.10	24.3	0.21
2S–5S	25.7	0.27	25.7	0.12

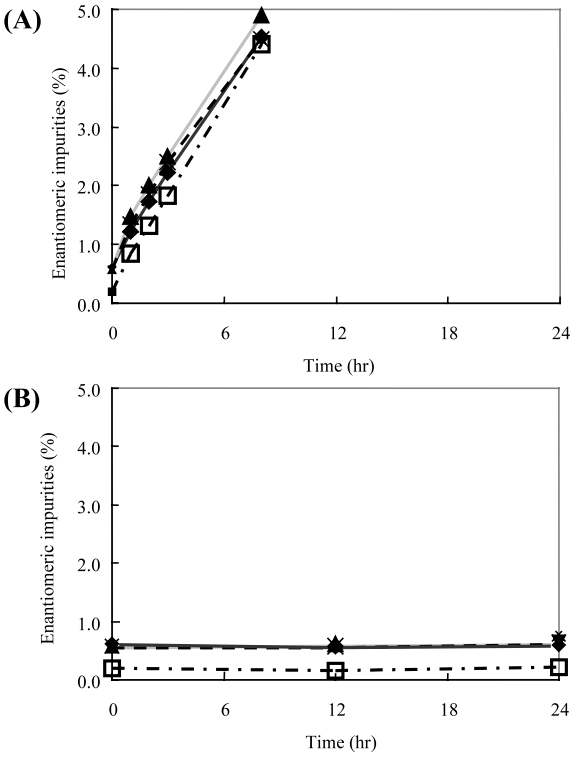


Fig. 10. Racemization of troglitazone stereoisomers in (A) methanol and (B) methanol–acetic acid (1000:1, v/v). (◆) 2R–5R, (□) 2S–5R, (▲) 2R–5S, (X) 2S–5S.

the standard addition method. Good separation and no other interference peaks were observed from the presence of placebo powder in the drug product. Three different amounts of troglitazone in the standard solution (~25, 50, 75 mg) which correspond to the 50, 100, and 150% of the troglitazone labeled claim amount, respectively, were added to 80 mg of placebo powder, and injected to test the recovery. Table 5 shows that recovery of each optical isomer was around 100% for all three concentrations. Repeatability studies were also carried out on samples of powdered drug product. Relative standard deviations of the percentage of each isomer (RR, SR, RS, SS) were 0.32, 0.09, 0.21, and 0.12, respectively, (n = 6), which were similar to analysis of the bulk substance, as shown in Table 3. The above results indicate that this proposed method can determine the optical isomers of troglitazone in the drug product accurately and precisely. A typical chromatogram of the troglitazone 100-mg tablet from this investigation is shown in Fig. 11.

Table 5
Recovery of troglitazone stereoisomers in pharmaceutical formulations

		Amount of troglitazone added (mg)		
		24.918	49.836	74.754
		Level 50%	100%	150%
Recovery (%)	2R–5R	100.4	100.4	101.2
	2S–5R	100.3	100.4	101.7
	2R–5S	100.4	100.6	101.3
	2S–5S	99.8	100.7	101.5

Using this method, the stability of the stereoisomer ratio of troglitazone in the drug product was evaluated. Table 6 shows the constancy of the stereoisomer ratio of troglitazone 200-mg tablet under several storage conditions. The stereoisomer ratios were proved to be unchanged for heat, humidity and light. Also this chiral separation method was applied for checking the stereoisomer

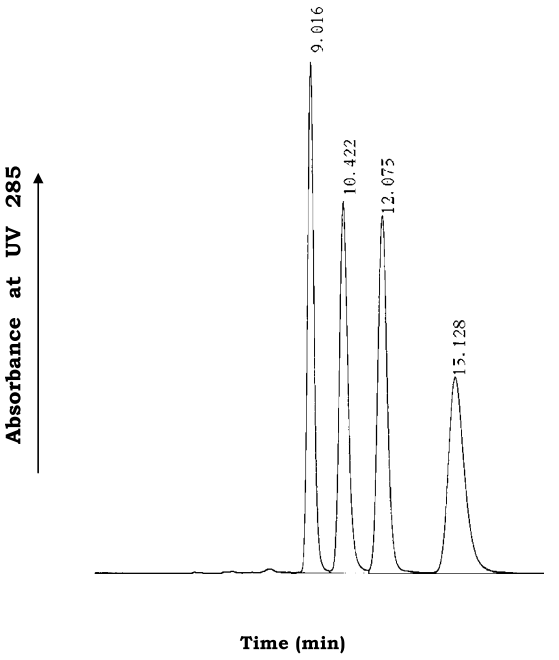


Fig. 11. HPLC chromatograms of troglitazone stereoisomers in the 100-mg tablets.

Table 6
Constancy of stereoisomer ratio of troglitazone in drug product in stability testing

Storage conditions	25 °C/60% RH		40 °C/75% RH		70 °C		40 °C/53% RH		Light irradiation	
Period	39 months		6 months		4 weeks		6 months		1.2 million lx × h	
Container	PTP/Al[a]		PTP/Al		PTP/Al		PTP/Al		Open glass bottle	
	Before	After	Before	After	Before	After	Before	After	Before	After
2R–5R	24	24	24	23	24	24	24	24	24	24
2S–5R	27	26	27	28	27	27	27	26	27	27
2R–5S	26	26	26	26	26	25	26	26	26	26
S-5S	23	24	23	23	24	23	24	24	24	23

[a] PTP package in aluminum bag with desiccant.

Table 7
Constancy of stereoisomer ratio of troglitazone in manufacturing process of the 200-mg tablets

Stereoisomers	Bulk substance	In-process materials			
		Solid dispersion	Blend for slugging powder	Tablet granules	Tablets
2R–5R	24	23	23	24	24
2S–5R	26	28	28	28	26
2R–5S	26	26	26	25	26
2S–5S	24	23	23	23	24

ratio in the in-process materials of the drug product. Table 7 shows the constancy of the stereoisomer ratio in the manufacturing process of the troglitazone 200-mg tablet. This manufacturing process was proved to have an ability to keep the stereoisomer ratio in the product.

4. Conclusion

The newly developed reversed-phase HPLC method has superior separation to the other HPLC methods evaluated in this study. And it is a simple method with good linearity, precision and detection sensitivity for determination of troglitazone stereoisomers. This method is a reversed-phase separation mode with short measurement time, 20 min per sample. So new possibilities of application are expected such as the stereoselective solubility study in aqueous solution or the quantitative analysis of stereoisomers in biological samples. Furthermore, this method is applicable and useful for evaluation of troglitazone stereoisomers in pharmaceutical formulations and in-process materials.

References

[1] E.J. Ariëns, Eur. J. Clin. Pharmacol. 26 (1984) 663–668.
[2] E.J. Ariëns, Med. Res. Rev. 6 (1986) 451–466.
[3] E.J. Ariëns, E.W. Wuins, Clin. Pharmacol. Ther. 42 (1987) 361–363.
[4] T. Yoshioka, T. Fujita, T. Kanai, Y. Aizawa, T. Kurumada, K. Hasegawa, H. Horikoshi, J. Med. Chem. 32 (1989) 421–428.
[5] T. Yoshioka, Y. Aizawa, T. Fujita, K. Nakamura, K. Sasahara, H. Kuwana, T. Kinoshita, H. Horikoshi, Chem. Pharm. Bull. 39 (1991) 2124–2125.
[6] T. Fujiwara, S. Yoshioka, T. Yoshioka, I. Ushiyama, H. Horikoshi, Diabetes 37 (1988) 1549–1558.
[7] S. Suter, J. Nolan, P. Wallace, B. Gumbiner, J. Olefsky, Diabetes Care 15 (1992) 193–203.
[8] J. Lambropoulos, A.B. Bergholdt, J. Pharm. Biomed. Anal. 24 (2000) 251–258.

[9] H.K. Jajoo, N.V. Rao Mamidi, K. Kasiram, A.S. Prakash, V.V. Swaroop Kumar, P. Bheema Rao, V. Bhushan, S. Subramaniam, J. Chromatogr. B Biomed. Sci. Appl. 707 (1998) 241–246.
[10] C.M. Loi, E.J. Randinitis, A.B. Vassos, D.J. Kazierad, J.R. Koup, A.J. Sedman, J. Clin. Pharmacol. 37 (1997) 1114–1120.
[11] A. Shibukawa, T. Sawada, C. Nakao, T. Izumi, T. Nakagawa, J. Chromatogr. A 697 (1995) 337–343.
[12] N. Cohen, R.J. Lopresti, G. Saucy, J. Am. Chem. Soc. 101 (1979) 6710–6716.
[13] K. Vyas, A. Sivalakshmidevi, C. Prabhakar, G.O. Reddy, Acta Crystallogr. C55 (1999) 411–413.
[14] K. Kobayashi, H. Fukuhara, T. Hata, Anal. Sci. 16 (2000) 443–444.
[15] Application Guide for Chiral Column Selection, second ed., Daicel Chemical Industries Ltd, Tokyo, pp. 38–50.
[16] Y. Okamoto, Y. Kaida, J. Chromatogr. 666 (1994) 403–419.
[17] H.Y. Aboul-Enein, M.R. Islam, J. Liq. Chromatogr. 13 (1990) 485–492.
[18] M. Yamamoto, M. Masaki, H. Nohira, Chirality 2 (1990) 280–283.
[19] H. Horikoshi, T. Yoshioka, T. Kawasaki, K. Nakamura, N. Matsunuma, K. Yamaguchi, K. Sasahara, Annu. Rep. Sankyo. Res. Lab. 46 (1994) 1–57.