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Physical property of troglitazone, an equal mixture of four stereoisomers

Nobuyuki Suzuki [a,*], Ko Kasahara [a], Hirokazu Hasegawa [b], Takao Kawasaki [a]

[a] *Product Development Laboratories, Sankyo Co., Ltd, 2-58, Hiromachi 1-chome, Shinagawa-ku, Tokyo 140-8710, Japan*
[b] *Process Development Laboratories, Sankyo Co., Ltd, 173, Nakahara-kamishuku, Hiratsuka-shi, Kanagawa 254-8560, Japan*

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Abstract

Troglitazone, an oral antidiabetic agent, is an equal mixture of four stereoisomers involving two chiral centers. In the present study, the physical property of troglitazone were investigated. The solid state of troglitazone drug substance is characterized as a simple physical mixture of two diastereomers, as shown by the two endothermic peaks caused by the melting of the RR/SS and the RS/SR forms by differential scanning calorimetry (DSC). In addition, the powder X-ray diffraction pattern includes peaks resulting from both the RR/SS and the RS/SR forms. The water adsorption of troglitazone drug substance is due to the presence of the RR/SS diastereomer, which adsorbs water as a monohydrate. The solubility of troglitazone and the diastereomers were increased and the solubility ratios of the stereoisomers were changed by quenching. Troglitazone was proved to be stable against heat and humidity by the ratio of the stereoisomers and from the solid state form indicated by the DSC results.

Keywords: Troglitazone; Diastereomer; Conglomerate; Hydration; Solubility; Solid state stability

1. Introduction

It is well recognized that polymorphism affects various pharmaceutically important physicochemical properties, such as stability, solubility, the dissolution rate, hygroscopicity, the crystal habit (shape), crystal hardness and tablet behavior (Bartolomei et al., 1999; Rustichelli et al., 2000; Bauer et al., 2001). Changes in certain of these physicochemical properties may ultimately affect the bioavailability of the drug (Kobayashi et al., 2000a). Therefore, investigation of the solid state characterization as well as the physical property of a drug substance is essential for drug development, especially for the rational design of the pharmaceutical dosage form and for the selection of appropriate conditions of manufacture and storage.

Thermal analysis and powder X-ray diffractometry (PXRD) are suitable and widely used techniques for the characterization of the solid state of the substance. Differential scanning calorimetry (DSC), which is a kind of thermal

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

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analysis, can supply valuable information regarding solid states with high sensitivity (McCauley and Brittain, 1995). If there are unusual exo/endothermic peaks in the DSC thermogram, the results have to be dealt with carefully from the point of view of polymorphism, hydration or any other variation in the solid state.

Troglitazone is a novel oral antidiabetic drug (Yoshioka et al., 1989, 1991) which 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 (Fujiwara et al., 1988; Suter et al., 1992). 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 as shown in Fig. 1, and is produced as a mixture of equal amounts of four optical isomers. There is a diastereomeric relationship between the isomers 2R-5S/2S-5R (RS/SR) and 2R-5R/2S-5S (RR/SS) in the troglitazone drug substance. In general, physicochemical properties of diastereomers including solubility are different (Grant and Brittain, 1995). For the evaluation of the properties of compounds having diastereomers such as troglitazone, the stereoisomer ratio in the substance has to be taken into account.

The objectives of this study are: (1) to identify and characterize the solid state of troglitazone drug substance using DSC and PXRD; (2) to explain the water adsorption mechanism, taking into consideration the solid state of the diastereomers; (3) to determine the respective solubility of the four isomers using a chiral HPLC method

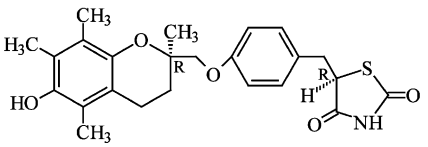
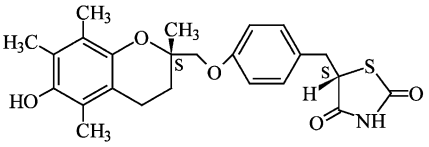
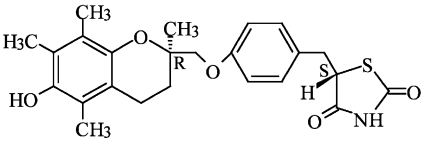
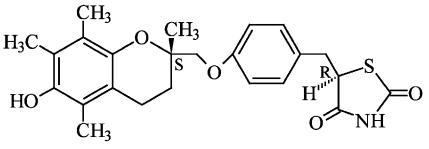
Diastereomer	Stereoisomer	Chemical Structure
RR/SS	2R-5R	
	2S-5S	
RS/SR	2R-5S	
	2S-5R	

Fig. 1. Chemical structures of troglitazone stereoisomers.

taking into consideration the solid state of the diastereomers; and (4) to identify other important property for drug development, such as stability.

2. Materials and methods

2.1. Materials

Troglitazone drug substance and the RS/SR and RR/SS forms were synthesized, described in the previous paper (Suzuki et al., 2002). A chiral HPLC column, Chiralcel OJ-R (4.6 mm i.d. × 150 mm, particle size: 5 μm), was purchased from Daicel Chemical Industries. Water was purified using a Milli-Q SP TOC system (Millipore Co., Ltd). Hydranal Aqualyte RS and Hydranal Coulomat CG for Karl-Fischer titration reagent were purchased from Riedel-de Haën GmbH. All other reagents and solvents were commercially available and of analytical grade.

2.2. Sample preparation of troglitazone

The physical mixture of troglitazone from the RS/SR and RR/SS forms (1:1) was made by softly gliding them together for 1 min.

The recrystallized substance after dissolving the RS/SR and RR/SS forms (1:1) was made by the same process as that of troglitazone drug substance. The acetone solution of the RS/SR and RR/SS forms was heated at 55 °C, and some water heated above 55 °C was added to this solution and the mixture was kept above 55 °C so as not to generate the drug precipitate. After confirming its dissolution, the solution was cooled gradually to slow crystallization. The mixture was cooled to 25 °C during a period of 130 min and then the substance was crystallized as much as possible. At around 25 °C, some more water was dropped at a constant rate into the mixture for 120 min, and then stirred for an additional 60 min to crystallize completely.

The amorphous forms of the troglitazone drug substance and the RS/SR and RR/SS forms were made by rapid cooling (quenching) of the completely melted substances. Troglitazone and the RS/SR and RR/SS forms were heated to melt on the furnace of the DSC, and then they were taken out of the furnace and immediately cooled by placing the sample onto a steel plate. To confirm the amorphous forms, the crystallinities were checked with a polarized light microscope (Nikon Opti-photo II, Japan).

All samples were dried with heating at 60 °C in vacuo for 15 h before the experiments.

2.3. Differential scanning calorimetry and thermogravimetric analysis

A DuPont thermal analyzer (Model 2000, New Castle, DE) was used to determine the DSC curves and weight loss with respect to temperature. The temperature axis and the cell constant of the DSC cell were calibrated with indium (10 mg, 99.999% pure, peak maximum at 166.6 °C). Each sample of troglitazone (~ 5 mg) was weighed into an aluminum pan and DSC studies were carried out in closed (sealed) pans with the temperature increased to 250 °C with a heating rate of 10 °C/min. Thermogravimetric analysis (TG) studies were carried out in open pans with the same conditions as for DSC.

2.4. Powder X-ray diffractometry

The PXRD patterns of troglitazone were determined at ambient temperature and atmosphere using a diffractometer (RINT2200, Rigaku Corp., Japan) with Cu kα radiation at 40 mA and 45 kV. Each sample was packed into an aluminum holder, and scanned with a diffraction angle of 2θ, increasing from 5 to 40°.

2.5. Chiral HPLC for the evaluation of four troglitazone stereoisomers

The concentrations of the troglitazone stereoisomers were determined by high-performance liquid chromatography (A Model HP1090, Agilent Technologies, USA), using the following HPLC conditions: The reversed-phase chromatography on a Chiralcel OJ-R was performed using a mobile phase composed of methanol–acetic acid (1000:1) at a flow rate of 0.5 ml/min. The column temperature was controlled at 25 °C, the injection volume

was 5 μl and detection was achieved at UV 285 nm. All of the stereoisomers were completely separated (Suzuki et al., 2002).

2.6. Water adsorption

The relative humidities (RH) for the water sorption isotherm at 25 °C were controlled using saturated salt solutions with known RH values (5, 33, 57, 67, 75%) in desiccators (Stokes, 1949).

About 300 mg of troglitazone substance and each of the diastereomers were each separately put into an open clear glass bottle, which was placed into the desiccator described above for 20 days until the water adsorption reached equilibrium. The samples before and after placing in the desiccator were weighed and the increase (% w/w) due to the water adsorption was calculated.

The initial water content, expressed as % w/w, was determined by Karl-Fischer titration using a Moisture Meter (Model AQ-5, Hiranuma Sangyo Co., Ltd, Ibaraki, Japan). Hydranal Aqualyte RS and Hydranal Coulomat CG were used as an anolyte and a catholyte, respectively. About 0.1 g of sample was weighed accurately, transferred to the titration vessel quickly, and dissolved in the anolyte.

2.7. Solubility

About 10 mg of troglitazone sample was put into a glass tube. Ten millilitre of 0.01 mol/l borate buffer, pH 9, was added and the suspensions were incubated at 37 °C. After 20 min, 1 ml of each suspension was filtered with PVDF filter Mini-Uniprep™ (5 mm in inside diameter, and pore size of 0.45 μm, Whatman Inc, USA). The filtrate was immediately measured by the chiral HPLC method described above to obtain the concentration of each stereoisomer. The concentrations of troglitazone were calculated by the peak area method using the three points of the calibration curve (2–200 μg/ml).

2.8. Solid state stability

The stability test was carried out under at the conditions of 40 °C/75% RH. Approximately 5 g of troglitazone substance was placed in a high-density polyethylene sack, which was placed in a 110 ml steel can. It was kept in a room controlled at 40 °C/75% RH for 6 months. The water content was determined by the Karl-Fischer titration method, the assay was by HPLC and the stereoisomer ratio was determined by the chiral HPLC method (Suzuki et al., 2002).

3. Results and discussion

3.1. Evaluation of four troglitazone stereoisomers with chiral HPLC

Troglitazone consists of the 4 stereoisomers derived from 2 asymmetric carbons. Its physical property can vary with change in the stereoisomer ratio by certain factors like crystallization. The stereoisomer ratios of troglitazone drug substance, the recrystallized sample and the physical mixture were measured by the chiral HPLC method described above. Quenched samples were also measured whose planes of polarization disappeared by checking with a polarized light microscope. Table 1 shows the list of the ratios of these samples indicating that chiral inversion rarely occurred during the melting-quenching, the recrystallization or the soft grinding. So the expected influence due to chiral inversion does not need to be considered in the following discussion.

3.2. DSC thermograms of troglitazone, RS/SR form and RR/SS form

The DSC method can be used to identify polymorphism by measuring exo/endotherms which characterize changes in morphology (McCauley and Brittain, 1995). Fig. 2 shows a typical DSC thermogram of troglitazone drug substance. There are two endothermic peaks at about 125 and 175 °C. Two diastereomers with different physicochemical properties exist in troglitazone. The RS/SR and the RR/SS forms, which are the diastereomers of troglitazone, were obtained in order to assign the two peaks. The physical mixture and the recrystallized substance were made from the two diastereomers. Fig. 3

Table 1
Stereoisomer ratios of various compositions and forms of troglitazone drug substance

Substance	Stereoisomer ratio (%)			
	RR	SR	RS	SS
Troglitazone drug substance (initial)	26	24	24	25
Quenched sample (amorphous)	26	25	25	25
Recrystallized sample	24	26	26	24
RS/SR form (initial)	0	50	50	0
Quenched sample (amorphous)	1	49	50	1
RR/SS form (initial)	51	0	0	49
Quenched sample (amorphous)	50	0	0	49
Physical mixture of RS/SR form and RR/SS form	26	24	24	25

shows the DSC thermograms of these substances. The melting thermograms of the RS/SR and RR/SS forms are shown on Fig. 3(A) and (B), respectively. In the physical mixture, at first the RR/SS form seemed to melt at around 130 °C, and then the broadening endothermic peak of the RS/SR form appeared with a melting point at around 175 °C which was lower than the endothermic melting point of the RS/SR form alone. This is due to the effect of the melted RR and SS molecules at the surface of the solid RS/SR form. Some endothermic detaching of the RS and SR molecules from the surface of the RS/SR form occur like the breaking of the lattice of the RS/SR

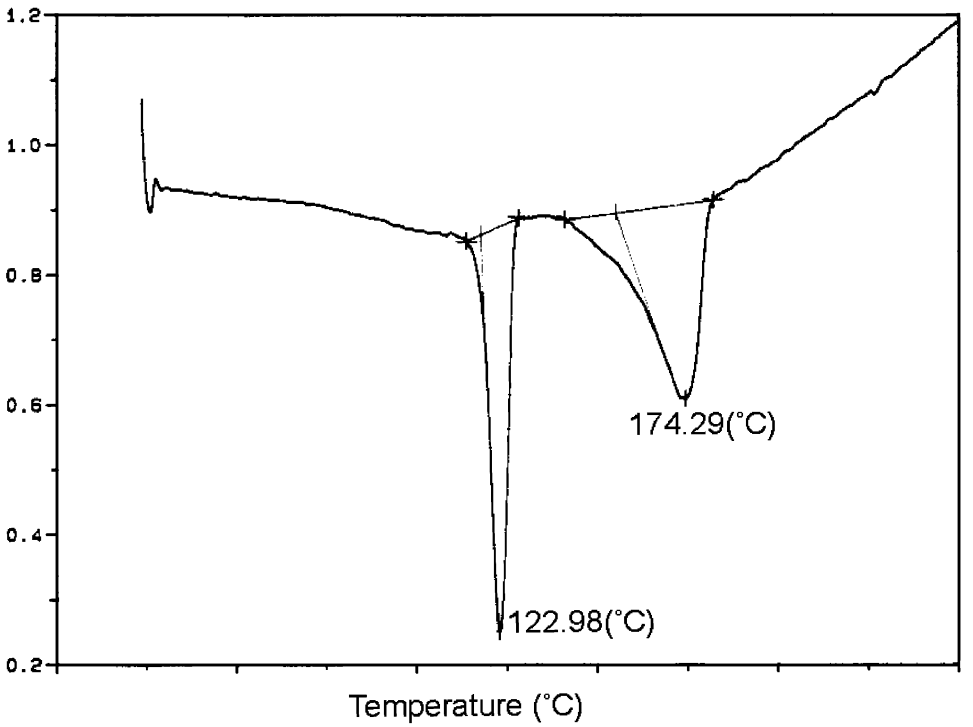


Fig. 2. DSC thermogram of troglitazone drug substance.

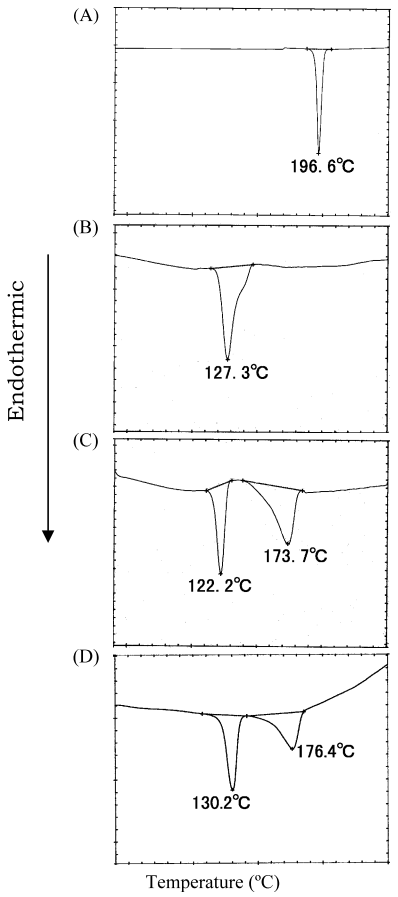


Fig. 3. DSC thermograms of troglitazone (A) RS/SR form; (B) RR/SS form; (C) recrystallized substance after dissolving RS/SR and RR/SS forms (1:1), and (D) physical mixture of RS/SR and RR/SS forms (1:1).

form. For example, the melted RR/SS works as a solvent at the surface of the RS/SR form and the RS and SR molecules are endothermically detached from the solid gradually at the lower temperatures from 140 to 180 °C. While the DSC thermograms of the physical mixture and the recrystallized substance were very similar, no other peaks corresponding to the existence of any polymorphism were found. The results indicate that the endothermic peak of troglitazone drug substance at 125 °C is due to the melting of the RR/SS form and the other peak at 175 °C is due to the melting and probably concurrent dissolving of the RS/SR form.

3.3. Powder X-ray diffraction

Fig. 4 shows the powder X-ray diffraction patterns of the recrystallized substance, the RS/SR form and the RR/SS form. Most peaks in the pattern of the recrystallized substance corresponded to the pattern of the RS/SR form or that of the RR/SS form. This just seems to be a superimposed pattern of the PXRD charts of the two diastereomers, and no other new diffraction peaks were observed. This suggests that no special formation such as a polymorph was found using this recrystallization method and there was no 'true racemate'-like paired unit of the diastereomers (Brittain, 1990). The diffraction patterns of troglitazone drug substance and the physical mixture were similar to that of the recrystallized substance. Thus, the results of the powder X-ray diffraction indicate that troglitazone drug substance consists of the crystalline RS/SR form and the crystalline RR/SS form with no interaction, like a 'conglomerate' (Brittain, 1990), supporting the DSC results described above.

3.4. Water adsorption

Water may be considered as being either bound or unbound, with the unbound portion generally being responsible for reactions requiring moisture as a reactant. And water can cause strong perturbations in the physical property of a solid. Therefore, it is important to clarify the water adsorption mechanism of a pharmaceutical drug substance (Zografi, 1988; Ahlneck and Zografi, 1990). Fig. 5 shows the adsorption isotherm of troglitazone, the recrystallized substance and the diastereomers in the range of 5–75% RH. The initial water content of troglitazone drug substance, the recrystallized substance, the RS/SR form and the RR/SS form were 0.8, 0.1, 0.0 and 0.1%, respectively. Although the RS/SR form does not show any hygroscopicity in this relative humidity range, the RR/SS form shows 3–4% water uptake in the same range. The recrystallized substance, which has a similar hygroscopic pattern to the troglitazone drug substance, shows a pattern intermediate between the hygroscopic patterns of the RS/SR form and the RR/SS form. So, the water adsorption of troglita-

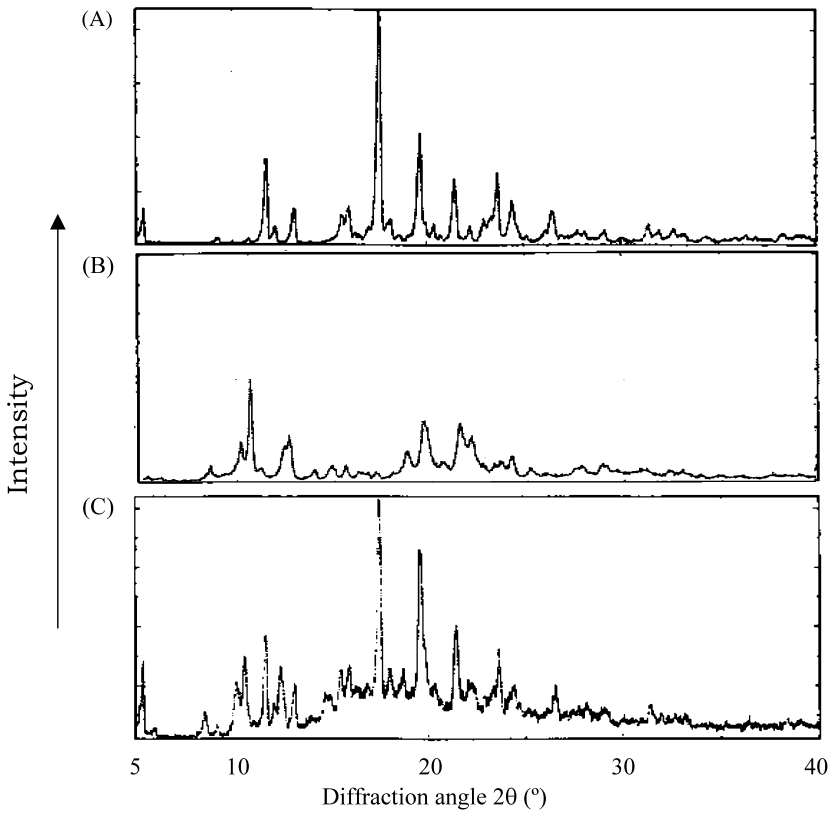


Fig. 4. PXRD patterns of troglitazone (A) RS/SR form; (B) RR/SS form, and (C) recrystallized substance after dissolving the RS/SR and RR/SS forms (1:1).

zone drug substance is due to the presence of the RR/SS form composing 50% of the substance.

The RR/SS form was analyzed by X-ray single crystal analysis and the existence of an RR/SS monohydrate has been reported (Kobayashi et al., 2000b). The amount of water uptake is close to the percentage of the monohydration (3.9%) in the RR/SS form. But the crystal structure was not changed in either the presence or absence of water, because no significant change except a slight increase in some peaks derived from the RR/SS form was observed in the PXRD patterns of the troglitazone recrystallized substance as shown in Fig. 6. These results indicate that there is no change in the crystal lattice around the water adsorption, and the adsorbed water is in a settled order under only 5% low relative humidity conditions.

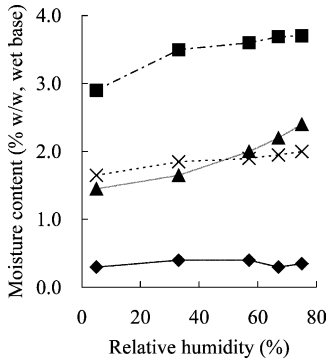


Fig. 5. Adsorption isotherms of troglitazone forms. (◆) RS/SR form; (■) RR/SS form; (×) recrystallized substance after dissolving the RS/SR and RR/SS forms (1:1), and (▲) troglitazone drug substance.

3.5. Solubility

The partition coefficient of troglitazone drug substance is high (Log P: 4.12, n-octanol/phos-

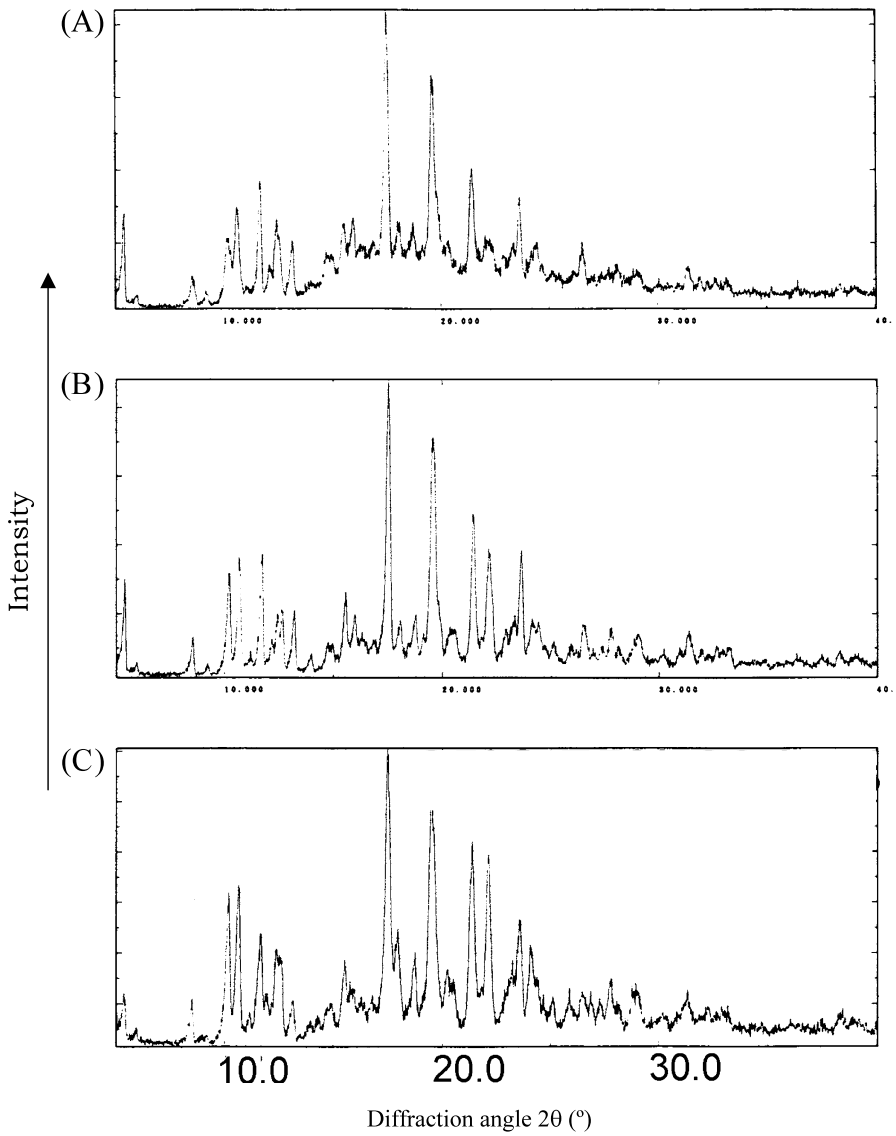


Fig. 6. Powder X-ray diffraction pattern of troglitazone recrystallized substance before and after water adsorption. (A) Initial; (B) after 20-day storage at 25 °C/5% RH; (C) after 20-day storage at 25 °C/75% RH.

phate buffer pH 5) indicating troglitazone is practically insoluble in water (Horikoshi et al., 1994), so the drug absorption into the body may be limited by the solubility. Therefore solubility is one of the key factors for the drug development of troglitazone.

Table 2 shows the stereoisomeric solubility of the troglitazone drug substance, the RS/SR form and the RR/SS form and quenched samples of these. The solubility of each enantiomer in the initial troglitazone substance is the same as the solubility in the diastereomeric forms (RS/SR form and RR/SS form). And the solubilities of RS/SR were slightly higher than those of RR/SS in all initial samples. This also suggests that both crystalline RS/SR form and crystalline RR/SS form exist in the troglitazone drug substance.

Table 2
Stereoisomer solubilities of various compositions and forms of troglitazone drug substance

Substance	Solubility (μg/ml)			
	RR	SR	RS	SS
Troglitazone drug substance (initial)	7.0	11.9	12.8	5.2
Quenched sample (amorphous)	125.4	87.3	89.0	120.3
RS/SR form (initial)		10.6	11.8	
Quenched sample (amorphous)		31.0	33.0	
RR/SS form (initial)	8.5			6.2
Quenched sample (amorphous)	46.7			46.4

Total solubility of troglitazone, the RS/SR form and the RR/SS form were improved by quenching. This may be caused by the differences of lattice energies among the RS/SR form, the RR/SS form and their amorphous forms, which are free from the restriction of the lattice. Thus, it makes sense that the amorphous forms were easier to dissolve than the crystals (Khankari et al., 1998; Hancock and Parks, 2000). Strictly, the solubility of each enantiomer in the quenched troglitazone drug substance was higher than the quenched RS/SR and RR/SS forms. Some prevention of crystallization causes keeping high solubility in the quenched troglitazone drug substance. For example, one diastereomer blocks attachment of the other diastereomer to its solid state surface, as well as an impurity prevents recrystallization.

These results indicate that making the drug amorphous can increase the solubility of the four stereoisomers and change the differences in their solubilities to each of the other enantiomers, unless any other solid state such as a polymorph were supplied in or as the troglitazone drug substance. This is important information for pharmacokinetic and drug formulation studies.

3.6. Solid state stability

The stability results of the troglitazone drug substance for 6 months storage at 40 °C/75% RH are shown in Table 3. Except for a slightly increase in water, no significant change was observed in the table and also in DSC thermograms. So the troglitazone drug substance was proved to be stable not only as a chemical structure but also in solid state form according to the DSC results, and retains the ratio of the stereoisomers.

Table 3
Stability of troglitazone drug substance

		Period			
		Initial	1 month	3 months	6 months
Assay (%)		99.3	98.9	99.3	99.1
Water (%)		0.9	1.2	1.2	1.6
Stereoisomer ratio (%)	RR	24	24	25	25
	SR	26	26	26	25
	RS	25	25	25	25
	SS	24	25	24	25

4. Conclusion

In this study, the solid state of troglitazone drug substance was characterized from the DSC and PXRD data used to determine the stereoisomeric ratio. Troglitazone exists as a simple physical mixture with two endothermic peaks caused by the melting of the RR/SS and the RS/SR forms, as shown by the DSC results. In addition, the PXRD pattern included peaks resulting from both the RR/SS and the RS/SR forms.

Although the RS/SR form does not exhibit any hygroscopicity, the RR/SS form shows 3–4% water uptake as a monohydrate in the range of 5–75% RH. And the water adsorption of troglitazone drug substance is due to the presence of the RR/SS diastereomer composing 50% of the drug substance.

The solubilities of the four stereoisomers in various solid states were determined. By quenching, the solubility of troglitazone and the diastereomers were improved, and the ratios of the solubility among the enantiomers of troglitazone were changed.

Troglitazone drug substance was not only proved to have no degradation, but also proved to be stable in solid state, retaining the ratio of the stereoisomers.

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