Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 46 (2008) 82–87
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Investigation of racemisation of the enantiomers of glitazone drug compounds at different pH using chiral HPLC and chiral CE

Babak Jamali [a,*], Inga Bjørnsdottir [b], Ole Nordfang [a], Steen Honoré Hansen [c]

[a] Protein Chemistry Diabetes, CMC Analysis & Formulation, R&D, Novo Nordisk A/S, Novo Alle, DK-2880 Bagsværd, Denmark

[b] DMPK & Bioanalysis, Preclinical Development, Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Måløv, Denmark

[c] Department of Pharmaceutics and Analytical Chemistry, Faculty of Pharmaceutical Science, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark

Received 2 April 2007; received in revised form 3 September 2007; accepted 4 September 2007
Available online 8 September 2007

Abstract

Drug enantiomers can have biologically distinct interactions within the biological system and consequently different pharmacological or toxicological effects. Development of a better and safer drug product may be considered if one of the enantiomers has a significantly better effect/side effect ratio than the other. Investigation of the single enantiomers in a racemic mixture could be valuable in order to investigate whether the single enantiomers demonstrate difference in pharmacological effect and/or fewer side effects versus the racemic mixture. In this context investigation of a possible racemisation of the pure enantiomers is very important.

In order to obtain the enantiomers of the racemic pioglitazone and the racemic rosiglitazone an HPLC method for chiral separation was developed. Using this method the R and S enantiomers were separated and the method was used to collect each enantiomer for investigation of racemisation process. The racemisation of the enantiomers of pioglitazone and rosiglitazone was investigated at pH 2.5, 7.4 and 9.3 using a chiral CE system. At pH 2.5 all enantiomers showed a slow racemisation. After 192 h (8 days) at 37 °C the ratio of the enantiomers in the mixture for all four isolated enantiomers was approximately 2 to 1 and after 1440 h (30 days) full racemisation was observed. The racemisation speed increased with increasing pH. At pH 7.4 the ratio of the enantiomers in the mixtures was approximately 2 to 1 already after 10 h. Full racemisation was observed within 48 h (2 days) at pH 7.4 and within 24 h at pH 9.3. These investigations have shown that it is possible to separate and isolate the enantiomers from a racemic mixture of glitazone drug substance and perform racemisation studies on each enantiomer.
© 2007 Elsevier B.V. All rights reserved.

Keywords: Pioglitazone; Rosiglitazone; Racemisation; pH; Chiral separation

1. Introduction

There is a great interest for glitazone drugs in the drug market for treatment of type 2 diabetes. Glitazones are potent insulin sensitizers [1,2]. Common for the chemical structure of glitazone compounds is the thiazolidinedione-ring with the chiral centre (Fig. 1). Since the chiral centre is attached next to a carbonyl group, the R enantiomer may convert to its S enantiomer or vice versa via keto-enol tautomerism (Fig. 1). Investigations have shown that the two glitazone drugs on the market (pioglitazone and rosiglitazone) are racemic mixtures [3]. However, in the literature there is no information about the enantiomers of the two glitazone drugs or on the stability of the single enantiomers with respect to their possible racemisation. Only one paper was found describing determination of the enantiomeric composition of R483 (a new glitazone drug under development) in plasma samples using chiral HPLC with electrospray tandem mass spectrometric detection [4].

Investigation of the single enantiomers in a racemic mixture may be valuable in order to investigate whether the single enantiomers demonstrate difference in pharmacological effect and/or fewer side effects versus the racemic mixture. Drug enantiomers can have biologically distinct interactions and consequently different pharmacological or toxicological effects [5–7]. Development of a better and safer drug product may be considered if one of the enantiomers has a significantly better effect/side

* Corresponding author. Tel.: +45 44434221.
E-mail address: bjam@novonordisk.com (B. Jamali).

0731-7085/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2007.09.004

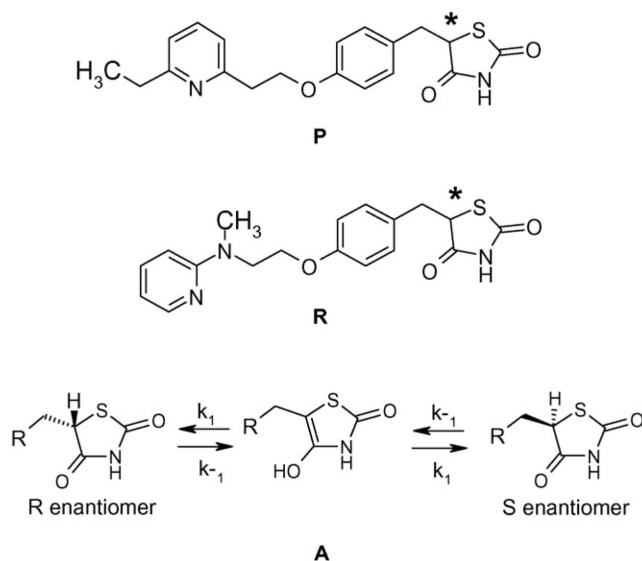

Fig. 1. Structure of pioglitazone (P) and rosiglitazone (R) and the proposed racemisation via keto-enol tautomerism (A). The * shows the chiral centre.

effect ratio than the other. However, in order to perform the necessary investigations for making decisions regarding this, it is required to test the enantiomers separately. So the pure enantiomers should either be synthesized (if possible) or purified from the racemic mixture using preparative separation methods. Stability of the single enantiomers in solution is one of the many issues that have to be investigated.

Prior to determination of the racemisation of the enantiomers of pioglitazone and rosiglitazone it was necessary to utilize preparative collection of the single enantiomers from racemic mixtures. A chiral capillary electrophoresis (CE) method [3] could be used for the separation of the enantiomers in the racemic mixture. However, sample collection from a CE system is very time consuming and not always optimal. A chiral high performance liquid chromatography (HPLC) system would be a preferable solution to this problem.

The aim of this paper is to demonstrate that it is possible to separate and collect enantiomers from a racemic mixture of glitazone drugs and perform racemisation or other studies on each enantiomer with respect to further investigations on these important potent insulin sensitizers and thus to a possible better treatment of diabetes.

This paper describes the development of the chiral HPLC for preparative isolation of the enantiomers from a racemic mixture of pioglitazone and rosiglitazone and the results of the racemisation studies of the enantiomers of each glitazone using a chiral CE method. The stability of enantiomers was studied in solution at three pH levels: low pH (pH 2.5), approximately physiologic pH level (pH 7.4) and high pH (pH 9.3).

2. Experimental

2.1. Chemicals

Purified water (water) was obtained from an in-house Milli-Q system (Millpore, Molsheim, France), Pioglitazone (obtained as HCl salt, from Jiangsu Wujin Hutang Secondary Fine Chemical Plant, China), Rosiglitazone (obtained as maleate salt, hydrate salt, from Sun Pharmaceutical Industries Ltd., India). All chemicals were obtained in the stated quality from manufacturers as follows.

2.1.1. For HPLC

Acetonitrile (ACN) HPLC grade (Merck, Darmstadt, Germany), methanol 99% (Merck, Darmstadt, Germany), ethanol 99% (Merck, Darmstadt, Germany), acetic acid 99% (Merck, Darmstadt, Germany), sodium perchlorate 98% (Sigma, St. Louis, USA), Chiralcel OJ, 250 mm × 4.6 mm i.d., Daicel (Chiral Tech., Ilkirch-Cedex, France). Mobile phase: 50 mM sodium perchlorate and 0.1 % (v/v) acetic acid in ethanol:methanol (5:95, v/v).

2.1.2. For CE

Five molars of sodium hydroxide (NaOH) (Bie & Berntsen, Copenhagen, Denmark). Sulfobutylether-β-cyclodextrin from (Advasep, Lenexa, USA purity 100% anhydrate, CyDex (SB-β-CD)), dimethyl-β-cyclodextrin (Cyclolab, Budapest, Hungary, 98 % purity (DM-β-CD)), sodium dihydrogen phosphate–monohydrate (Merck, Darmstadt, Germany). The capillary was an 80.5 cm (72.0 cm to detector) 50 μm inner diameter ("Extended Light Path Capillary") Agilent Technologies capillary HP part no. G1600-62232.

Preparation of solutions for CE: 25 mM sodium phosphate buffer pH 8.0:690 mg sodium dihydrogen phosphate–monohydrate was dissolved in approximately 180 ml water, the pH value was adjusted to 8.0 with 5 M sodium hydroxide and finally the solution was diluted to 200 ml with water. Running buffer for CE (0.7% w/v DM-β-CD, 0.2 % w/v SB-β-CD in 25 mM phosphate buffer pH 8.0):200 mg SB-β-CD and 70 mg DM-β-CD were dissolved in 10.0 ml 25 mM sodium dihydrogen phosphate pH 8.0 and filtered through a filter (approx. 0.45 μm). Nine millilitres of this solution was added with 1.0 ml of ACN. The CE vials were filled with approximately 1.0 ml of running buffer.

2.1.3. For racemisation studies

Ortho phosphoric acid 85% (Merck, Darmstadt, Germany), 50 mM borate buffer pH 9.3 (Agilent Technologies, Waldbronn, Germany), boric acid (Merck, Darmstadt, Germany).

Buffers for racemisation studies: 25 mM phosphate buffer pH 2.5 and pH 7.4 was prepared as follows: in a 1000 ml measuring flask 2882 mg of 85% ortho phosphoric acid was added to approximately 800 ml Milli-Q water. The pH value was adjusted to 2.5 or 7.4 with 5 M sodium hydroxide and Milli-Q water was added to the mark. Twenty-five millimolars of borate buffer pH 9.3 was prepared as follow: 50.00 ml, 50 mM borate buffer and pH 9.3 (Agilent Technologies, Waldbronn, Germany) was added approximately 30 ml Milli-Q water and the pH was checked and/or adjusted using 0.1 M sodium hydroxide (or boric acid) to 9.3. The solution was then transferred to a 100 ml measuring flask and Milli-Q water was added to the mark.

2.2. Instrumentation

2.2.1. HPLC

HPLC was carried out using a Waters (Milford, USA) Alliance HPLC system equipped with a 2587 Dual λ Absorbance UV Detector set at 220 nm. The HPLC pump mode was isocratic with a flow of 0.5 ml/min and a run time of 35 min. The injection volume was 10 μl. Column temperature was 25 °C. Data acquisition and signal processing was performed using Waters Millennium 32 (Version 4.00, Waters, Milford, USA).

2.2.2. CE

Capillary electrophoresis was performed according to a previously published method [3] using an Agilent Technologies ^{3D}CE system (Walbronn, Germany). Data acquisition and signal processing were performed using Agilent Technologies ^{3D}CE ChemStation (rev. A.06.03, Agilent Technologies).

UV detection was performed at 225 nm. The auto sampler was operated at room temperature (approximately 21 °C). In the evaluation of the racemisation corrected peak areas (peak area divided by migration time) was used.

New capillaries were flushed with 1.0 M NaOH for 20 min followed by 20 min with 0.1 M NaOH and 10 min with water. Used capillaries were conditioned for 10 min with 0.1 M NaOH and then flushed with Milli-Q water for 10 min. Capillaries were rinsed after each sample run with 0.1 M NaOH and then with water (flushed for 1 min with each). Hydrodynamic injection at 40 mbar for 5.0 s (approx. 4 nl) was used. The voltage was +30 kV (50 μA approximately). The temperature around the capillary was 30 °C. The run time was 15 min. The resolution (R_s) between pioglitazone enantiomers was 3.5 (migration time of approximately 9.9 and 10.2 min). The resolution between rosiglitazone enantiomers was 3.7 (migration time of approximately 9.9 and 10.2 min).

2.3. Sample preparation

2.3.1. HPLC

The sample concentration was 170 μg/ml for each pioglitazone and rosiglitazone calculated as the non-ionized molecule. The samples for HPLC analysis were dissolved in methanol.

2.3.2. CE

The samples for CE analysis were prepared as follows: to one aliquot collected fraction from chiral HPLC containing a mixture of ethanol and methanol (5:95, v/v) with 0.1 % (v/v) acetic acid and 50 mM sodium perchlorate, four aliquots of buffer solution (pH 2.5, 7.4 or 9.3) was added.

2.4. Racemisation studies

The racemisation studies were performed in solution at three pH values: pH 2.5 using 25 mM phosphate buffer, pH 7.4 using 25 mM phosphate buffer and pH 9.3 using 25 mM borate buffer.

Each single enantiomer (four in all) was isolated by chiral HPLC and the enantiomeric purity of the collected samples was tested by CE immediately after collection. Samples was then added the appropriate buffer solution as described under sample preparation. The peak purity was found to be at least 99% before addition of buffer solution. The samples were kept at 37 °C during the racemisation studies. The racemisation rates in samples were determined using the chiral CE system. The samples were analyzed just after the addition of the buffer and after 24, 48, 96, 144, 192, 720 and 1440 h (pH 2.5), after 2, 4, 6, 8, 10 and 48 h (pH 7.4) and after 1, 2, 3, 4, 5 and 24 h (pH 9.3).

3. Results and discussion

3.1. Development of the chiral HPLC method

In order to investigate the dependence of pH on the racemisation rate of the enantiomers of the glitazone compounds there was a need for preparative isolation of the enantiomers in proper amounts. Pioglitazon and rosiglitazon are racemic mixtures and during literature search only two methods [3,4] for the separation of the enantiomers of glitazone compounds could be found. One of these [3] involved a chiral CE method for separation of the enantiomers of pioglitazon and rosiglitazon. However, preparative CE is very difficult and tedious to perform and therefore usage of a preparative chiral HPLC system would be a better solution for preparation of the necessary amounts of the enantiomers.

As cyclodextrins had shown to be useful for CE separation [3] of these compounds, two HPLC columns containing cyclodextrins: Cyclobond I 2000 and Cyclobond I 2000 DM were tested firstly using both a polar organic phase (acetonitrile/methanol (95:5) with 0.2%, v/v triethyl amine and 0.3%, v/v acetic acid added) and using reversed phase conditions (acetonitrile/10 mM phosphate buffer pH 3.0 95:5, v/v). However, no separation was obtained for any of the racemic mixtures of glitazones and this is in fact in accordance with observations from CE experiments [3] where no or only very little separation could be achieved using only one cyclodextrin.

A number of other available chiral HPLC columns were screened using their standard recommended LC conditions. Chiral columns such as Chiral-AGP, Chiral HAS, Chiral CBH, ChiroSil® but also Ceramospher™ chiral columns (Chiral RU-1, Chiral RU-2 and Chiral CD-Ph) were tested but no separation was obtained for any of the racemic mixtures of glitazones.

Another chiral column which also was tested during the development work was Chiralcel OJ column. Suitable mobile phases recommended by column manufacturer for this column are typically hexane/isopropanol mixtures mostly as 90:10 (v/v) but hexane/ethanol mixtures may also be used [8,9]. Other suitable mobile phase modifiers are *N*,*N*-diethylamine (for basic compounds) and trifluoroacetic acid (for acidic compounds) [8,9]. *N*-diethylamine and trifluoroacetic acid were therefore tested with 90:10 (v/v) hexane/isopropanol and hexane/ethanol. Unfortunately no separation was achieved with Chiralcel OJ column with the recommended systems. During the screening work a new paper on glitazone separation was published by Susuki et al. [10]. This publication described a chiral HPLC method which was applied for pharmacokinetic and metabolism studies on troglitazone. The chiral HPLC method was a Chiralcel

OJ column (150 mm × 1.0 mm i.d., particle size 5 μm, Daicel Chemical Ind., Tokyo, Japan) using a mobile phase consisting of methanol and acetic acid (1000:1, v/v) containing 50 mM of lithium perchlorate ($LiClO_4$) at a flow rate of 20 μl/min. The LC system with the Chiralcel OJ column described by Susuki et al. [10] was therefore tested for separation of the enantiomers of pioglitazone and rosiglitazone with the two exceptions: sodium perchlorate was used instead of lithium perchlorate and the Chiralcel OJ column was a 250 mm × 4.6 mm i.d. (Daicel).

No separation was achieved for neither pioglitazone nor rosiglitazone enantiomers. An optimization of the LC conditions was therefore needed in order to reach separation of the enantiomers of both compounds. During the optimization process the concentration of acetic acid (0.1%, v/v) and perchlorate ion (50 mM sodium perchlorate) was kept constant.

Different organic solvents such as ethanol, isopropanol and acetonitrile were tested as replacement for methanol in the mobile phase containing acetic acid (0.1%, v/v) and sodium perchlorate ion (50 mM). When using ethanol in the mobile phase a minor separation was observed but the peaks were too broad and the retention time increased. Therefore, it was decided to try a polar organic LC mode using a combination of methanol and other organic solvents. Methanol (with 0.1%, v/v acetic acid and 50 mM sodium perchlorate) was then tested in combination with ethanol, isopropanol and acetonitrile. The tested combinations were as follows (methanol/organic solvent in v/v): 50/50, 75/25, 90/10 and 95/5. The best chiral separation was achieved by combination of methanol with ethanol (95/5). The combination 97.5/2.5 was also tested but no further improvement was observed. The effect of flow was also tested from 0.2 to 1.0 ml/min. Flow 0.5 ml/min resulted in good efficiency and selectivity with Gaussian-curved peaks. The loadability of the system was tested from 5 to 50 μg on-column. This experiment showed that loading of more than 25 μg of the compound resulted in broad peaks and loss of resolution indicating system overload.

The optimization resulted in a LC system operating with the mobile phase methanol:ethanol (95/5, v/v) with 0.1% (v/v) acetic acid and 50 mM sodium perchlorate added using a flow rate of 0.5 ml/min. For each injection 25 μg compound was injected onto the column. The separation of the racemates into the enantiomers by chiral HPLC can be seen in Fig. 2. Each enantiomer was isolated and collected in solution sufficient for the racemisation studies. Unfortunately, baseline separation was not achieved for pioglitazone and rosiglitazone enantiomers at the applied concentration (preparative), but by selection of proper fractions, enantiomers of high purity (>99%) could be collected and used for investigation of the racemisation at different pH values. The separation of the isolated enantiomers of the pioglitazone and rosiglitazone by chiral CE using a dual chiral selector system can be seen in Fig. 3. Although it was not possible in this study to assign the absolute configuration of each enantiomer it was observed that the enantiomer with highest affinity to the stationary phase in HPLC being a carbohydrate also was the enantiomer that had the highest affinity to the chiral selectors (being a cyclic polysaccharide) in CE (thus providing

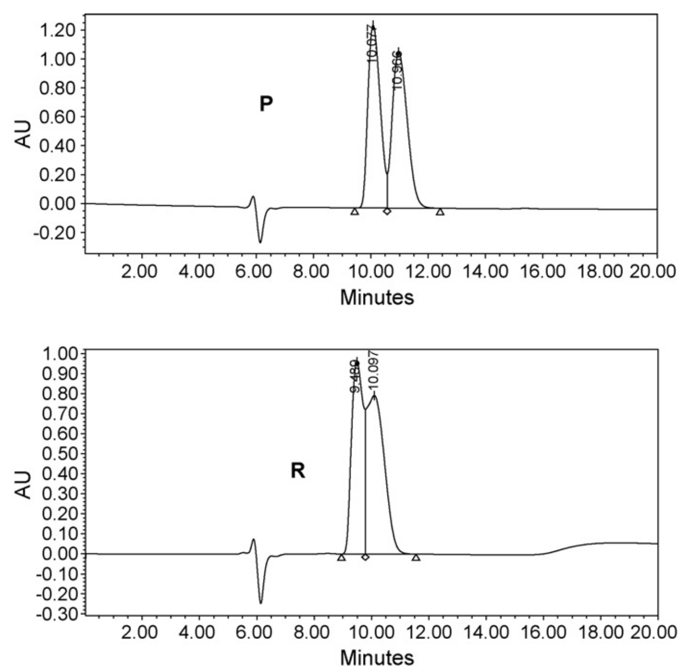

Fig. 2. Separation of the enantiomers of pioglitazone (P) and rosiglitazone (R) by chiral HPLC (Chiralcel OJ, 250 mm × 4.6 mm i.d., mobile phase: 50 mM sodium perchlorate and 0.1% (v/v) acetic acid in ethanol:methanol 5:95 (v/v), 0.5 ml/min, column temperature at 25 °C and UV detection at 220 nm). Baseline separation was not achieved for rosiglitazone enantiomers at the applied concentration (preparative).

the shortest migration time). This leads to the following cross-identification between the two separation systems: HPLC peak 1 (first eluted peak in the HPLC system) = CE peak 2 (peak with the highest migration time) and vice versa. This was verified for both pioglitazone and rosiglitazone.

3.2. Racemisation in different pH

The effect of pH on the racemisation was studied at pH 2.5, 7.4 and pH 9.3 using chiral CE for determination of each enantiomer [3]. The results obtained can be seen in Table 1.

At pH 2.5 all enantiomer shows a relatively slow racemisation. After 24 h a ratio between enantiomers of approximately 95 to 5 was reached. After 720 h (30 days) the ratio is approximately 60 to 40 and after 1440 h (2 months) full racemisation was observed.

At pH 7.4 the speed of the racemisation process was faster. After 4 h an equilibrium showing a ratio of approximately 80 to 20 was observed, after 10 h an equilibrium of 70–30 was reached and within 48 h (2 days) full racemisation was obtained.

At pH 9.3 the speed of the racemisation process was much faster. Already after 2 h the ratio was approximately 85 to 15. After 5 h the ratio of approximately 70 to 30 was observed and full racemisation was observed within 24 h.

The racemisation of the enantiomers may involve at least one intermediate (Fig. 1) and as the racemisation ends up as a true racemic mixture it may be considered as a reversible first order reaction [11] and the data was therefore fitted to the rate equation

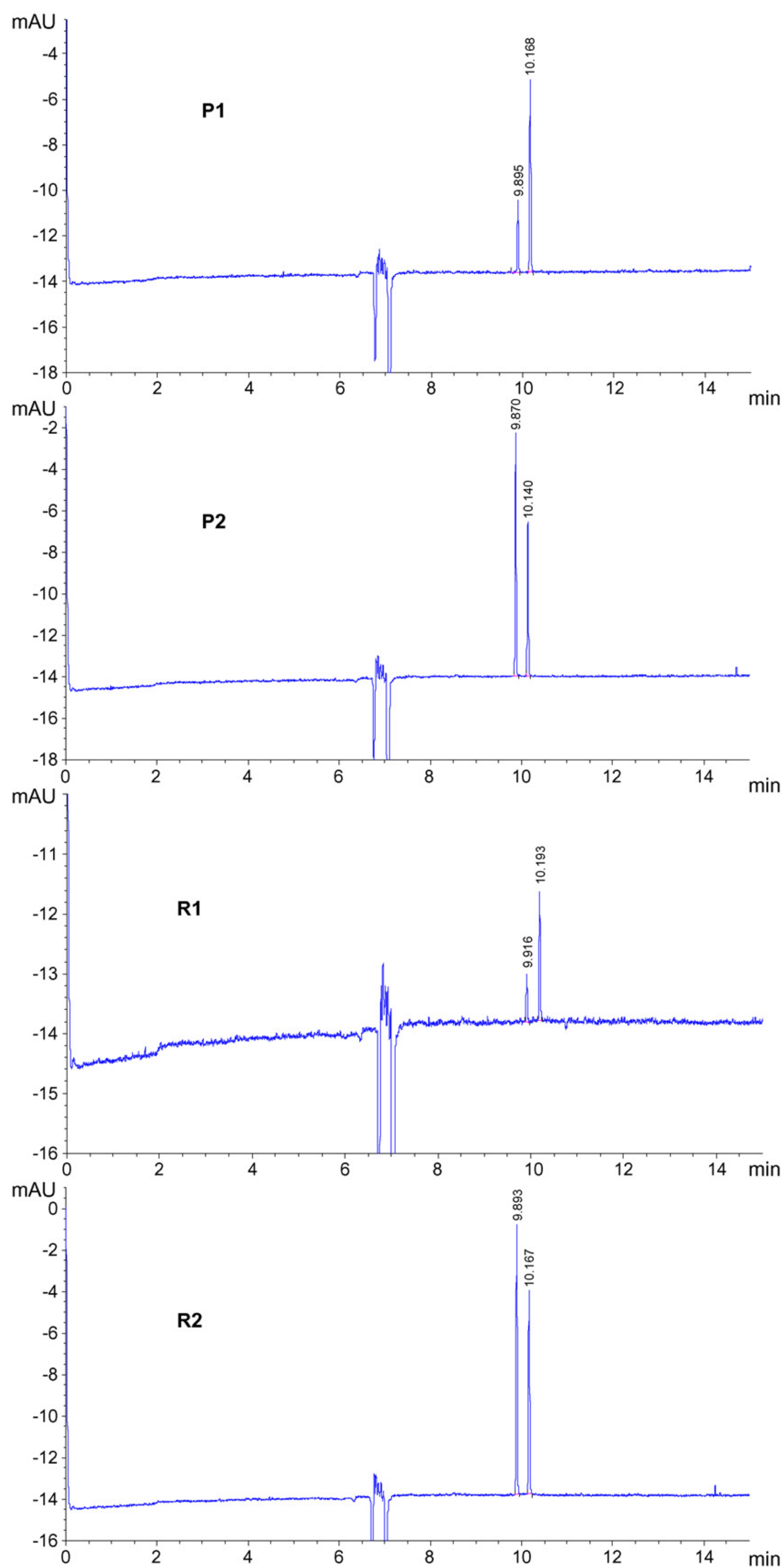

Fig. 3. An example of the separation of the enantiomers of pioglitazone enantiomers (P1: HPLC peak 1; P2: HPLC peak 2) and rosiglitazone enantiomers (R1: HPLC peak 1; R2: HPLC peak 2) at pH 2.5 day 8 by CE (80.5 cm capillary (72.0 cm to detector) 50 μm i.d. "Extended Light Path Capillary" Agilent Technologies; running buffer: 0.7% (w/v) DM-β-CD and 0.2 % w/v SB-β-CD in 25 mM sodium phosphate buffer pH 8.0, UV detection at 225 nm; auto sampler at room temperature (approximately 21 °C)).

Table 1
Enantiomer ratios (peak areas) as a function of pH and time

pH	Time (h)	Pioglitazone		Rosiglitazone	
		HPLC peak 1	HPLC peak 2	HPLC peak 1	HPLC peak 2
2.5	0	100:0	0:100	100:0	0:100
	24	94:6	5:95	92:8	4:96
	48	90:10	8:92	89:11	7:93
	96	84:16	11:89	79:21	9:91
	144	79:21	14:86	76:24	12:88
	192	74:26	18:82	73:27	14:86
	720	59:41	35:65	60:40	33:67
	1440	50:50	50:50	50:50	50:50
7.4	0	100:0	0:100	100:0	0:100
	2	82:18	11:89	91:9	13:87
	4	74:26	17:83	84:16	17:83
	6	69:31	21:79	79:21	23:77
	8	63:37	25:75	73:27	23:77
	10	60:40	26:74	70:30	28:72
	48	50:50	50:50	50:50	50:50
9.3	0	100:0	0:100	100:0	0:100
	1	81:19	3:97	80:20	3:97
	2	76:24	8:92	77:23	14:86
	3	70:30	16:84	69:31	19:81
	4	68:32	21:79	65:35	16:84
	5	65:35	23:77	63:37	24:86
	24	50:50	50:50	50:50	50:50

below:

$$\ln\left(\frac{c_A - c_A^e}{c_A^0 - c_A^e}\right) = -(k_1 + k_{-1})t$$

where c_A is the amount (%) of enantiomer in focus measured at time t, c_A^e the amount (%) of enantiomer in focus at the end of racemisation process, c_A^0 the amount (%) of enantiomer in focus measured at start of the process, k_1 and k_{-1} are the rate of racemisation and t is the time (h). The c_A^0 is 100 (%) and c_A^e is 50 (%). The above equation can therefore be expressed as

$$c_A - 50 = \exp(\ln 50 - kt)$$

and

$$t_{0.5} = \frac{\ln 2}{k}$$

From this the reaction rate and half-life ($t_{0.5}$) could be calculated (Table 2).

Racemisation takes place at all pH values investigated and the racemisation rate increase with increasing pH. The half-life of the enantiomers at physiological pH is about 4 h.

These studies showed that it is possible to separate and isolate the enantiomers from a racemic mixture of glitazone drugs and perform racemisation (or other) studies on each enantiomer and obtain information on the racemisation and stability of the enantiomers. The isolation process showed to be time demanding and have some limitations as the speed of the racemisation of the enantiomers in solution is relatively fast. Thus, the efficacy and safety of one enantiomer versus the other must be significantly better before there can be an interest for developing and producing a drug product with only one enantiomer.

Table 2
Racemisation rate and half racemisation time for enantiomers of pioglitazone and rosiglitazone at pH 2.5, 7.4 and 9.3

	Pioglitazone		Rosiglitazone	
	HPLC peak 1	HPLC peak 2	HPLC peak 1	HPLC peak 2
pH 2.5				
k (changes%/h)	−0.00228	−0.00159	−0.00206	−0.00144
$t_{0.5}$ (h)	304	435	337	482
pH 7.4				
k (changes%/h)	−0.17739	−0.18301	−0.18313	−0.18259
$t_{0.5}$ (h)	4	4	4	4
pH 9.3				
k (changes%/h)	−0.35723	−0.37189	−0.35598	−0.37098
$t_{0.5}$ (h)	2	2	2	2

4. Conclusion

A chiral HPLC method was developed for separation of the enantiomers in racemic mixtures of pioglitazone and rosiglitazone and was used to collect each enantiomer for further investigations.

The rate of racemisation of the isolated enantiomers of pioglitazone and rosiglitazone was investigated in solution at three pH values (2.5, 7.4 and 9.3) using a chiral CE system. The stability studies showed a relatively slow racemisation at pH 2.5 and faster racemisation at pH 7.4 and pH 9.3.

Acknowledgments

The excellent help and lab expertise of Lise-Lotte Sørensen and Gitte Cargan Theill and the support of Karsten Wassermann and Steen Guttorm Andersen during this work are gratefully acknowledged.

References

[1] C. Otto, M. Lehrke, B. Göke, Pharmacogenomics 3 (2002) 99–116.
[2] S. Mudaliar, R.R. Henry, Annu. Rev. Med. 52 (2001) 239–257.
[3] B. Jamali, G.C. Theill, L.L. Sørensen, J. Chromatogr. A 1049 (2004) 183–187.
[4] B. Lausecker, G. Fischer, J. Chromatogr. B 835 (2006) 40–46.
[5] E.J. Ariënes, W. Soudijin, P.B.M.W.M. Timmermans (Eds.), Stereochemistry and Biological Activity of Drugs, Blackwell, London, 1983.
[6] D.B. Campbell, Eur. J. Drug Metabol. Pharmacokinet. 15 (1990) 109–125.
[7] A.R. Fassihi, Int. J. Pharm. 92 (1993) 1–14.
[8] Instruction Manual for Chiralcel OJ columns, March 2003, http://www.chiral.fr.
[9] Chiralcel OJ columns, Instruction Sheet, DAICEL Chemical Industries Ltd. at http://www.daicel.co.jp/chiral/e/product/instruction/oj.html.
[10] N. Susuki, N. Miyashita, A. Kotani, F. Kusu, F. Kawasaki, J. Pharm. Biomed. Anal. 38 (2005) 155–161.
[11] K.A. Connors, Chemical Kinetics, VCH Publishers Inc., New York, USA, 1990, pp. 59–77 (chapter 3).