Journal of Pharmaceutical and Biomedical Analysis 48 (2008) 934–939

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

An improved LC–ESI–MS–MS method for simultaneous quantitation of rosiglitazone and *N*-desmethyl rosiglitazone in human plasma

Grace O'Maille[a,*], Sudhakar M. Pai[a], Xiaolu Tao[a], George T. Douglas Jr.[b], Rand G. Jenkins[b]

[a] *Akros Pharma Inc., Clinical Pharmacology, 302 Carnegie Center, Suite 300, Princeton, NJ 08540, USA*
[b] *PPD Development, 2244 Dabney Road, Richmond, VA 23230, USA*

A R T I C L E I N F O

Article history:
Received 12 February 2008
Received in revised form 29 July 2008
Accepted 3 August 2008
Available online 13 August 2008

Keywords:
Rosiglitazone
N-desmethyl rosiglitazone
Human plasma
LC–ESI–MS–MS

A B S T R A C T

A fast, sensitive, and selective method for the simultaneous quantitation of rosiglitazone and *N*-desmethyl rosiglitazone in human plasma, using rosiglitazone-d_4 and *N*-desmethyl rosiglitazone-d_4 as the respective internal standards, has been developed and validated. The analytes in human plasma (50 μL sample aliquot) were isolated through supported liquid/liquid extraction (SLE) and separated by isocratic HPLC over a 3-min period. The precursor and product ions were detected by ESI–MS–MS with multiple reaction monitoring (MRM) in a triple quadrupole mass spectrometer. For both rosiglitazone and *N*-desmethyl rosiglitazone, the lower limit of quantitation (LLOQ) was 1.00 ng/mL, and the quantitation range was 1.00–500 ng/mL (with an average correlation coefficient >0.9990). The intra-assay and inter-assay precision had a maximum %CV of 9.37%, and the accuracy had a maximum %difference from theoretical of 12.7%. This method was applied to a clinical study where 16 healthy volunteers were administered a single dose of 4.0 mg rosiglitazone. The pharmacokinetic parameters of rosiglitazone and *N*-desmethyl rosiglitazone were consistent with the results reported in the literature.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

Rosiglitazone (Avandia®) is a widely prescribed oral antidiabetic agent for the treatment of Type 2 diabetes mellitus [1,2], and it is primarily metabolized by cytochrome P450 2C8 (CYP2C8) enzyme, where *N*-desmethyl rosiglitazone is one of the major metabolites [3,4]. Rosiglitazone is a sensitive 2C8 substrate and its systemic exposure (plasma AUC) is increased by 2-fold or more when co-administered with gemfibrozil (a CYP2C8 inhibitor) [5]. Therefore, plasma concentrations of rosiglitazone and *N*-desmethyl rosiglitazone can provide information regarding the effect of concomitantly administered drugs on *in vivo* CYP2C8 enzyme activity [6,7].

While specific and sensitive LC–MS–MS methods have been reported in the literature for the quantitation of both rosiglitazone and *N*-desmethyl rosiglitazone in plasma [6–8]; however, these methods are only semi-quantitative for the *N*-desmethyl metabolite. Plasma concentrations of the metabolite could only be estimated in arbitrary units due to lack of an appropriate reference standard. For example, a recent publication presented a HPLC–ESI–MS–MS method for the quantitation of rosiglitazone and *N*-desmethyl rosiglitazone [8]; however, although an expected and potentially selective mass transition ($[M+H]^+$ precursor ion/product ion pair) for *N*-desmethyl rosiglitazone was monitored, the method was validated for the quantitation of rosiglitazone alone.

In the current paper, we describe a specific and sensitive HPLC–ESI–MS–MS method for the simultaneous quantitation of rosiglitazone and *N*-desmethyl rosiglitazone in human plasma. The method utilized a short (3-min) chromatographic separation and was validated with a Lower Limit of Quantitation (LLOQ) of 1.00 ng/mL, and a linear range of 1.00–500 ng/mL. The quantitation was performed using peak areas ratios of the detected analyte peaks to those of isotopically labeled (deuterated) internal standards. Additionally, this method was developed, validated and applied to clinical samples from 16 healthy volunteers administered a single oral dose of 4 mg of rosiglitazone, and the pharmacokinetic parameters were calculated and presented.

2. Experimental

2.1. Chemicals and materials for validation study

Rosiglitazone, CAS #122320-73-4, was purchased from Molcan Corporation (Ontario, Canada) with a purity of >98%. Rosiglitazone-d_4, synthesized by PPD with a purity of >98%, was used as the internal standard for Rosiglitazone. *N*-desmethyl rosiglita-

* Corresponding author. Tel.: +1 609 919 6121; fax: +1 609 919 9575.
E-mail address: omaille@akrospharma.com (G. O'Maille).

0731-7085/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2008.08.001

zone and its internal standard *N*-desmethyl rosiglitazone-d_4 were purchased from SynFine Research (Ontario, Canada) at purities >98%. Human plasma, tripotassium EDTA, was purchased from Biochemed (Charleston, South Carolina, U.S.A.). Isolute, SLE +, 96-well plates (product number 820-0200-PO1) from Biotage (Charlottesville, Virginia, U.S.A.) were used for analyte extraction. Analytical grade acetonitrile, ethyl acetate, hexane, isopropyl alcohol, and methanol were purchased from VWR Scientific Products (West Chester, Pennsylvania, U.S.A.). Ethylene glycol, formic acid (~98%), and trifluoroacetic acid were purchased from Sigma (Sigma–Aldrich, St. Louis, Missouri, U.S.A.).

2.2. Standard solutions preparation and stability evaluation

Stock solutions of rosiglitazone and *N*-desmethyl rosiglitazone were prepared in methanol and methanol acidified with 0.1% formic acid, respectively, at nominal concentrations of 100 μg/mL. An internal standard working solution was prepared in methanol at nominal rosiglitazone-d_4 and *N*-desmethyl rosiglitazone-d_4 concentrations of 500 ng/mL. Solutions were stored at −20 °C or colder. The analyte stability in solution for all stock solutions and internal standard solutions were demonstrated for up to 6 h of storage at room temperature, and for storage at −20 °C, rosiglitazone, rosiglitazone-d_4, *N*-desmethyl rosiglitazone, and *N*-desmethyl rosiglitazone-d_4 were demonstrated to be stable for 356 days, 173 days, 69 days and 31 days, respectively.

2.3. Calibration standards and quality controls preparation and acceptance criteria

Calibration standards were prepared in human plasma in duplicate and containing tripotassium EDTA, at nominal rosiglitazone and *N*-desmethyl rosiglitazone concentrations of 1.00, 1.75, 3.50, 12.0, 40.0, 150, and 500 ng/mL. The correlation coefficient of the calibration curve for each validation run must be at least 0.990. Quality control pools were prepared in the same matrix and in duplicate, with nominal analyte concentrations of 1.00, 2.50, 6.00, 24.0, 80.0, and 375 ng/mL. After thorough mixing, aliquots of each pool were stored frozen in polypropylene tubes at −20 °C (or colder). For a run to be accepted, the acceptance criteria of at least 67% of the batch QCs and at least 50% of the QC replicates from each level tested must quantitate within accuracy acceptance limits. The acceptance limit for the QC LLOQ (lowest level QC, prepared at the same concentration as the LLOQ sample) is ±20% and the limit for all other levels is ±15% of their respective theoretical analyte concentrations.

2.4. Sample preparation

A 50.0-μL sample aliquot was diluted with 100 μL of water, fortified with 25.0 μL of 500 ng/mL internal standard working solution and loaded onto a 96-well Isolute SLE+ (supported liquid/liquid extraction) plate. After a 5-min equilibration period, 3 μL × 350 μL of 60:40 hexane/ethyl acetate (v/v) were passed through the SLE+ plate to elute the analytes into a 96-well collection plate and the extract was evaporated under a nitrogen stream at room temperature. The remaining residue was reconstituted with 500 μL of 50:50 acetonitrile/10 mM ammonium acetate with 0.02% trifluoroacetic acid (v/v). The final extract was analyzed via HPLC with MS/MS detection.

2.5. HPLC conditions

HPLC separation was carried out using an Allure PFP Propyl column (5 μm particle size, 2.1 mm × 50 mm) from Restek Corporation (Product No. 9169552) and an HP 1100 Series pump from Agilent Technologies. Isocratic conditions of 40% A and 60% B were used, where mobile phase A was composed of 10 mM ammonium acetate with 0.02% trifluoroacetic acid, and B was acetonitrile. The separation was performed at room temperature at a flow rate of 0.300 mL/min, with an approximate run time of 3.5 min. An injection volume of 25.0 μL was used for all sample extracts.

2.6. MS/MS conditions

A Sciex API 3000, triple quadrupole LC/MS/MS mass spectrometer (Applied Biosystems) operating in positive TurboIonSpray® (ESI) ionization mode with MRM detection was used for all analysis. The ion source temperature was set at 450 °C, the ion spray voltage was 1500 V, and the electron multiplier (CEM) was 2400 V. A nitrogen collision gas flow (CAD) of 10.0 L/min, curtain gas flow (CUR) of 8.00 L/min, nebulizer gas flow (NEB/GS1) of 8.00 L/min, and auxiliary gas flow (AUX/GS2) of 8.00 L/min were used. The mass spectrometer was calibrated using a polypropylene glycol calibration solution. The dwell times were set to 100 ms and the collision energies were set to 36 eV for the detection of all four analytes: rosiglitazone, rosiglitazone-d_4, *N*-desmethyl rosiglitazone and *N*-desmethyl rosiglitazone-d_4. The monitored precursor ions for rosiglitazone and its d_4 internal standard were m/z 358.1 and 362.1, respectively, and a common product ion of m/z 135.1 was detected at a retention time of ~1.18 min. Monitored precursor ions for *N*-desmethyl rosiglitazone and its d_4 internal standard were m/z 344.1 and 348.1, respectively, with a common product ion of m/z 121.1 detected at a retention time of ~1.05 min.

3. Results

3.1. Validation of the method

3.1.1. Linearity and calibration

For each standard curve, eight calibration standards (at 1.00, 1.75, 3.50, 12.0, 40.0, 150, 400, and 500 ng/mL) were analyzed in duplicate over the nominal concentration range of 1.00–500 ng/mL; three standard curves were set up in three different days. A quadratic, 1/concentration weighted, least squares regression algorithm was used to plot the peak area ratio of the appropriate analyte to its internal standard versus concentration. The mean correlation coefficient from the three standard curves was >0.9990 for both rosiglitazone and *N*-desmethyl rosiglitazone. The back-calculated values and reproducibility from each level of the calibration curve resulted in a percent coefficient of variation (%CV) range of 1.49–8.00%. Results from the three standard curves are shown in Table 1.

3.1.2. Limit of quantitation

A lower limit of quantitation of 1.00 ng/mL for rosiglitazone and *N*-desmethyl rosiglitazone was established in the validation; representative chromatograms shown with those of internal standards are illustrated in Fig. 1.

3.1.3. Precision and accuracy

Precision and accuracy were evaluated by analyzing quality control (QC) pools prepared at 1.00, 2.50, 6.00, 24.0, 80.0, and 375 ng/mL, where the lowest QC level is the same as the LLOQ for this method. Precision was expressed as the percent coefficient of variation (%CV) of each QC pool. Accuracy was measured as the percent difference from theoretical. The individual results for each of the precision and accuracy evaluations are shown in Table 2.

Intra-assay precision and accuracy were evaluated for each quality control pool by multiple analyses (n = 6) of the QC pool during three separate validation runs. The intra-assay data resulted in a

Table 1
Average back-calculated calibration standards for rosiglitazone and *N*-desmethyl rosiglitazone, where mean concentration values, standard deviation (S.D.) and percent coefficient of variation (%CV) are shown

	Theoretical concentration (ng/mL)							
	1.00	1.75	3.50	12.0	40.0	150	400	500
Rosiglitazone								
Mean (n = 6)	1.02	1.75	3.47	11.9	39.5	152	397	502
S.D.	0.0414	0.0697	0.0976	0.380	1.44	2.29	17.3	25.5
%CV	4.06	3.98	2.81	3.19	3.66	1.51	4.37	5.08
N-desmethyl rosiglitazone								
Mean (n = 6)	1.03	1.77	3.39	12.2	38.7	151	403	497
S.D.	0.0825	0.0787	0.103	0.808	0.577	5.47	12.0	29.1
%CV	8.00	4.44	3.04	6.64	1.49	3.63	2.98	5.86

maximum coefficient of variation (%CV) of 9.37% and maximum percent difference from theoretical of ±12.7% for both rosiglitazone and *N*-desmethyl rosiglitazone.

Inter-assay precision and accuracy were evaluated by analyzing six replicates of each QC level in three runs. The inter-assay data (n = 18) resulted in a maximum %CV of 6.65% and maximum percent difference from theoretical of ±9.55% for both rosiglitazone and *N*-desmethyl rosiglitazone.

Parallelism of diluted study samples was also investigated. The ability to analyze samples with insufficient volume for a full aliquot was validated by analyzing six replicate 24.0 ng/mL QCs as 5-fold dilutions. The ability to dilute samples originally above the upper

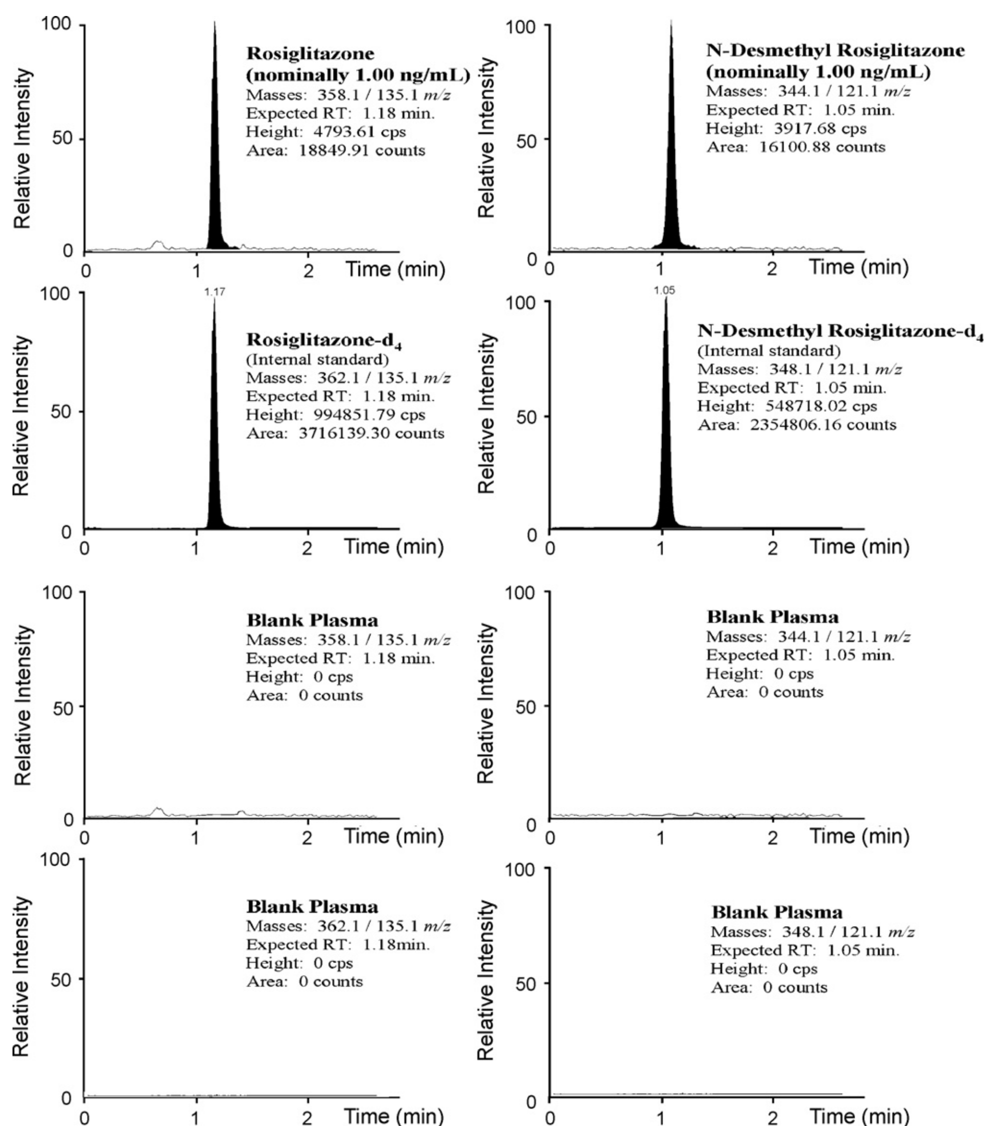

Fig. 1. Lower limit of quantitation standard (rosiglitazone and *N*-desmethyl rosiglitazone), nominally 1.00 ng/mL. The relative intensity of the chromatograms of blank plasma samples are scaled the same as the corresponding spiked samples.

Table 2
Intra-assay and inter-assay precision and accuracy of quality control samples for rosiglitazone and *N*-desmethyl rosiglitazone, where percent coefficient of variation (%CV) and percent difference from theoretical (%Δ) are shown

	Theoretical concentration (ng/mL)					
	1.00	2.50	6.00	24.0	80.0	375
Rosiglitazone						
Inter-assay						
%CV ($n=6$)	5.83	6.21	3.96	3.07	7.59	5.33
%Δ ($n=6$)	6.84	3.33	−3.35	−5.67	−6.33	−3.22
Intra-assay						
%CV ($n=18$)	6.56	5.54	3.60	4.20	5.09	5.04
%Δ ($n=18$)	2.80	−0.143	−2.50	−2.96	−4.20	−0.656
N-desmethyl rosiglitazone						
Inter-assay						
%CV ($n=6$)	9.37	8.41	7.64	6.52	5.64	4.63
%Δ ($n=6$)	12.7	4.17	−3.08	−4.67	−5.9	1.29
Intra-assay						
%CV ($n=18$)	6.65	6.21	6.03	6.53	4.75	3.55
%Δ ($n=18$)	9.55	2.95	−0.346	0.231	−2.03	1.67

limit of the calibration range was validated by analyzing six replicate 1000 ng/mL QCs as 5- and 10-fold dilutions. For rosiglitazone, the individual %CV for 24.0 ng/mL (5-fold dilution), 1000 ng/mL (5-fold dilution) and 1000 ng/mL (10-fold dilution) were 6.32%, 3.36%, and 4.06%, respectively, where the corresponding percent difference from theoretical are −6.31%, −1.95%, and 2.38%, respectively. For *N*-desmethyl rosiglitazone, the %CV, in the same order, were 3.60%, 2.10%, and 3.69%, with corresponding percent difference from theoretical values at −5.68%, −2.14%, and 5.23%. Therefore, an overall maximum %CV of 6.32% and maximum percent difference from theoretical of ±6.31% was obtained for both rosiglitazone and *N*-desmethyl rosiglitazone in all three experimental setups.

Based on these results, the current method shows acceptable precision and accuracy for both analytes.

3.1.4. Recovery

Extraction recovery of the analytes from human plasma was evaluated by comparing analyte responses of pre-extraction spiked samples to those of post-extraction spiked samples. Matrix-related ionization effects were evaluated by comparing analyte responses of post-extraction spiked samples to those of external standards (non-matrix prepared samples) representing 100% recovery. An overall maximum %CV of 7.05% and 10.7% were obtained for rosiglitazone and *N*-desmethyl rosiglitazone, respectively. The individual %recovery values from the recovery evaluation experiments are shown in Table 3.

3.1.5. Stability

Freeze/thaw stability was evaluated by analyzing low- and high-level quality controls (2.50 ng/mL and 375 ng/mL) that were subjected to four freeze/thaw cycles. Samples were thawed at room temperature. The stability data is acceptable for a QC level if the %CV of the replicate determination does not exceed 15.0% and the accuracy of the mean value is within ±15% of the theoretical value for that pool. Based on this acceptance criteria, no apparent abnormalities associated with up to four freeze/thaw cycles were observed as indicated by %CV and %difference from theoretical of <4.00% for both rosiglitazone and *N*-desmethyl rosiglitazone.

Analyte stability in thawed matrix was evaluated by allowing a set of low- and high-level quality controls to thaw and remain at room temperature for 24 h prior to extraction and analysis. No apparent abnormalities associated with storage for up to 24 h at room temperature were observed.

Reinjection reproducibility was evaluated by analyzing calibration standards and quality controls that were extracted and injected as part of one run and stored at room temperature prior to and during reanalysis in another run. No apparent abnormalities associated with reinjection of sample extracts were observed.

Post-preparative extract stability was evaluated by analyzing quality controls that were extracted, injected as part of one run and stored at room temperature for approximately 80 h prior to and during reinjection in another run 3 days later. These samples were quantified versus the original calibration curve in the run that was analyzed on the day of extraction. No apparent abnormalities associated with post-preparative storage for up to 80 h at room temperature were observed.

Analyte stability in frozen matrix was evaluated over the course of the validation by analyzing the intra-assay QCs versus freshly prepared calibration standards in each core validation run. The quality controls ($n=6$) analyzed demonstrated analyte stability in frozen matrix for a period of 8 days at −20 °C.

The %CV and %difference from the theoretical for the replicate ($n=6$) low- and high-level stability QCs were all <6.00% under the stability test conditions described above.

3.1.6. Specificity

Human plasma samples, containing tripotassium EDTA, from six individuals were extracted and analyzed for rosiglitazone, *N*-desmethyl rosiglitazone, and their internal standards. There were no significant chromatographic peaks detected at the mass transitions and expected retention times of the analytes or their internal standards, which would interfere with quantitation.

Additional specificity samples, fortified with rosiglitazone and *N*-desmethyl rosiglitazone at 2.00 ng/mL, were prepared from six individual human plasma lots and analyzed to evaluate potential matrix suppression effects. A seventh lot of human plasma samples, prepared from the matrix used to prepare the calibration standards and quality controls were analyzed as a control. To be acceptable, greater than 67% of the replicates for a lot must quantitate within ±15% of the theoretical value for five out of six fortified specificity sample lots. All %CV and %difference from theoretical were <6.00%, indicating that no significant matrix suppression effects were present that could compromise the sensitivity or accuracy of the assay.

3.1.7. Cross-analyte interference

Aliquots of blank human plasma were fortified with only one analyte or internal standard and analyzed in triplicate. There were

Table 3
General extraction recovery and matrix-related ionization effects for rosiglitazone and *N*-desmethyl rosiglitazone, where analyte response and internal standard response (Int. Std. Response) were compared between pre- and post-extraction fortified samples, and those of post-extraction fortified samples and external standards, which are free of human plasma

	Theoretical Concentration (ng/mL)		
	2.50	24.0	375
Rosiglitazone			
Pre-extraction fortified vs. post-extraction fortified			
Analyte response %recovery	78.6%	80.4%	80.7%
Int. Std. response %recovery	85.2%	82.8%	78.3%
Post-extraction fortified vs. external standard			
Analyte response %nominal response	97.9%	91.7%	98.9%
Int. Std. response %nominal response	94.0%	93.1%	103%
N-desmethyl rosiglitazone			
Pre-extraction fortified vs. post-extraction fortified			
Analyte response %recovery	71.6%	71.8%	71.1%
Int. Std. response %recovery	76.9%	73.1%	71.5%
Post-extraction fortified vs. external standard			
Analyte response %nominal response	94.9%	101%	96.9%
Int. Std. response %nominal response	93.1%	106%	97.5%

no chromatographic peaks detected at the mass transitions or expected retention times of the unfortified components.

3.2. Application of the method

3.2.1. Clinical application

The commercially available 4 mg rosiglitazone tablet Avandia® (rosiglitazone maleate, GlaxoSmithKline) was used in the clinical study conducted as part of a drug interaction trial to assess CYP2C8 activity. The study was conducted according to applicable regulations and guidelines for Good Clinical Practice (i.e., ICH, FDA). Three-and-a-half hours after the start of a standardized breakfast, a single dose of 4 mg rosiglitazone was administered orally to 16 male volunteers, between the ages of 18 and 65, with a body mass index (BMI) between 18 and 32 kg/m^2, and determined to be healthy based on pre-study medical history, physical examination, vital signs, 12-lead ECG, and clinical laboratory tests. Blood samples for pharmacokinetic assessments were collected in Vacutainer® brand tubes containing tripotassium EDTA as the anticoagulant at the following time-points: immediately before dosing (0 h; predose) and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 14, 24, and 48 h after drug administration. The plasma was separated by centrifugation (4 °C, 1100 × g, 10 min) and stored frozen at −20 °C, until analysis. The samples were analyzed using the validated method within the established stability period. Mean plasma concentration–time profiles of rosiglitazone and *N*-desmethyl rosiglitazone are shown in Fig. 2, and the pharmacokinetic parameters are summarized in Table 4. The pharmacokinetic parameters (C_{max}, T_{max}, AUC_∞, and $t_{1/2}$) were calculated by non-compartmental methods, using WinNonlin version 5.1 (Pharsight Corporation, Mountain View, CA, U.S.A.).

Rosiglitazone was quantifiable in all subjects and attained peak concentrations (C_{max}) at approximately 0.5 h; thereafter,

Table 4
Pharmacokinetic parameters (mean ± S.D.) following a single oral dose of rosiglitazone (4 mg) administered to healthy male volunteers

Parameter	Rosiglitazone (n = 16)	*N*-desmethyl rosiglitazone (n = 16)
T_{max} (h)[a]	0.50 (0.50–1.55)	6.00 (4.00–8.02)
C_{max} (ng/mL)	332.5 ± 59.7	72.09 ± 8.75
$t_{1/2}$ (h)	4.02 ± 0.68	16.90 ± 4.79
AUC_∞ (ng h/mL)	1388 ± 359	2370 ± 756
CL/F (L/h)	1.809 ± 0.426	N/A

[a] T_{max} represents median (minimum–maximum).

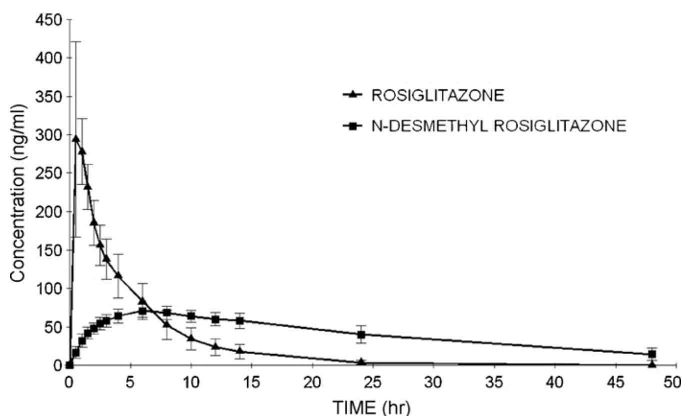

Fig. 2. Mean plasma concentration–time profiles of rosiglitazone and *N*-desmethyl rosiglitazone in healthy male subjects after a 4 mg single oral dose of rosiglitazone maleate. The error bars represent standard deviations at each time-point.

the concentrations declined with a terminal half-life of 4 h. *N*-desmethyl rosiglitazone concentrations were quantifiable in individual subjects beginning at the 0.5 h post-dose time-point; peak concentrations were attained at approximately 6 h post-dose; the concentrations declined thereafter with a half-life of 17 h. The indices of systemic exposure (C_{max} and AUC_∞) showed low inter-subject variability. For rosiglitazone and *N*-desmethyl rosiglitazone, the coefficient of variation for C_{max} was 18% and 12%, respectively; the corresponding values for AUC_∞ were 27% and 32%.

4. Discussion

The quantitation of rosiglitazone has previously been reported in methods using HPLC and ultraviolet (UV) detection or fluorescence detection [9–15]. These HPLC with UV/fluorescence detection methods are straightforward, selective, and fast, with a LC separation of typically <15 min; however, large sample volumes (~1 mL) are typically required, and these methods only reported the concentration of rosiglitazone. Hruska and colleagues extended the study on the metabolism of rosiglitazone and used a modified method with HPLC coupled with MS–MS detection [7]; although the concentrations of rosiglitazone and *N*-desmethyl rosiglitazone were reported, the metabolite concentrations were reported in arbitrary units (U/L) because an authentic *N*-desmethyl

rosiglitazone calibration curve was not used. Cox and colleagues reported a comprehensive analysis of the absorption, disposition and metabolism of rosiglitazone [4]; while the report presented metabolite identification by mass spectrometry, the quantitation was done by measuring the radioactivity after administration of [^{14}C]rosiglitazone. The most recent published method on rosiglitazone is an HPLC–ESI–MS–MS method by He et al. [8]; it is important to note that the article by He et al. also contains a detailed list and brief discussion of many other recently published analytical methods for rosiglitazone concentration determination [8]. The method developed by He et al. is sensitive and selective; however, the quantitation was validated for rosiglitazone alone [8]. As mentioned in the introduction section, the method by He et al. is similar to other articles that included the *N*-desmethyl metabolite, that although the chromatographic peak for *N*-desmethyl rosiglitazone was described, quantitation data were not provided [8].

The pharmacokinetic parameters for rosiglitazone and *N*-desmethyl rosiglitazone in the current study are consistent with those reported previously in the literature [6,7]. It is important to note that in the previous studies, because estimated *N*-desmethyl rosiglitazone concentrations were reported in arbitrary units, C_{max} and AUC_{∞} were also reported in arbitrary units [6,7]. In the current study, we successfully quantitated the *N*-desmethyl metabolite concentrations in plasma using a calibration curve prepared from a reference standard and a stable isotope-labeled internal standard. Thus, the parameters C_{max} and AUC_{∞} are reported in actual units, which therefore assure the accuracy and precision of the evaluation of the pharmacokinetic parameters of the *N*-desmethyl metabolite. Insofar as *N*-desmethyl rosiglitazone concentrations are concerned, agreement of data from the current study with those reported in the literature lends credibility to the latter.

In conclusion, we report an improved HPLC–ESI–MS–MS method that was validated for the simultaneous quantitation of rosiglitazone and *N*-desmethyl rosiglitazone in human plasma. In addition to the excellent sensitivity and selectivity of the method, the 3-min runtime is the fastest reported assay for simultaneous analysis and quantitation of rosiglitazone and *N*-desmethyl rosiglitazone. The method can be used to reliably quantify rosiglitazone and its *N*-desmethyl metabolite in human plasma samples from clinical trials, such as those involving drug–drug interactions, which affect *in vivo* CYP2C8 enzyme activity.

Acknowledgements

The authors thank Matt Halquist and Neal Simmons, formerly of PPD.

References

[1] J. Patel, E. Miller, R. Patwardhan, et al., Diabetes 47 (1998) A17.
[2] M. Ashiya, R.E.T. Smith, Nat. Rev. Drug Discov. 6 (2007) 777–778.
[3] S.J. Baldwin, S.E. Clarke, R.J. Chenery, Br. J. Clin. Pharmacol. 48 (1999) 424–432.
[4] P.J. Cox, D.A. Ryan, F.J. Hollis, A.-M. Harris, A.K. Miller, M. Vousden, H.C. Ley, Drug Metab. Dispos. 28 (2000) 772–780.
[5] Drug Interaction Studies. Study design, data analysis, and implications for dosing and labeling. Draft Guidance, September 2006, FDA (CEDR, CBER). http://www.fda.gov/cder/guidance/6695dft.htm.
[6] M. Niemi, J.T. Backman, P.J. Neuvonen, Clin. Pharmacol. Ther. 76 (2004) 239–249.
[7] M.W. Hruska, J.A. Amico, T.Y. Langaee, R.E. Ferrell, S.M. Fitzgerald, R.F. Frye, Br. J. Clin. Pharmacol. 59 (2005) 70–79.
[8] J. He, Y.F. Hu, L.F. Duan, Z.R. Tan, L.S. Wang, D. Wang, W. Zhang, Z. Li, J. Liu, J.H. Tu, Y.M. Yao, H.-H. Zhou, J. Pharm. Biomed. Anal. 43 (2007) 580–585.
[9] A.M. Muxlow, S. Fowles, P. Russell, J. Chromatogr. B Biomed. Sci. Appl. 752 (2001) 77–84.
[10] R.N.V.S. Mamidi, M.R. Chaluvadi, B. Benjamin, M. Ramesh, K. Katneni, A.P. Babu, J. Bhanduri, N.M.U. Rao, R. Rajagopalan, Arzneim. -Forsch. 52 (2002) 560–564.
[11] T. Radhakrishna, J. Satyanarayana, A. Satyanarayana, J. Pharm. Biomed. Anal. 29 (2002) 873–880.
[12] M.W. Hruska, R.F. Frye, J. Chromatogr. B 803 (2004) 317–320.
[13] K.-A. Kim, J.-Y. Park, Biomed. Chromatogr. 18 (2004) 613–615.
[14] B.L. Kolte, B.B. Raut, A.A. Deo, M.A. Bagool, D.B. Shinde, J. Chromatogr. Sci. 42 (2004) 70–73.
[15] R.S. Pedersen, K. Brosen, F. Nielsen, Chromatographia 62 (2005) 197–201.