Journal of Chromatography B, 877 (2009) 1951–1956

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Short communication

Simultaneous quantification of rosiglitazone and its two major metabolites, N-desmethyl and *p*-hydroxy rosiglitazone in human plasma by liquid chromatography/tandem mass spectrometry: Application to a pharmacokinetic study

Kwon-Bok Kim[a], Dong Jun Lee[b], Chang-Woo Yeo[a], Jae-Gook Shin[a,b], Soo Kyung Bae[a,b,*]

[a] *Department of Pharmacology and PharmacoGenomics Research Center, Inje University College of Medicine, Busan, South Korea*
[b] *Department of Clinical Pharmacology and Clinical Trial Center, Inje University Busan Paik Hospital, Busan, South Korea*

ARTICLE INFO

Article history:
Received 15 January 2009
Accepted 1 May 2009
Available online 13 May 2009

Keywords:
Rosiglitazone
N-desmethyl rosiglitazone
p-Hydroxy rosiglitazone
Human plasma
LC–MS/MS

ABSTRACT

We present a simple, rapid, and sensitive liquid chromatography (LC)–tandem mass spectrometry (MS/MS) method for the simultaneous quantification of rosiglitazone and its two major metabolites via CYP2C8/9, N-desmethyl and *p*-hydroxy rosiglitazone, in human plasma. The procedure was developed and validated using rosiglitazone-d_3 as the internal standard. Plasma samples (0.1 ml) were prepared using a simple deproteinization procedure with 0.2 ml of acetonitrile containing 40 ng/ml of rosiglitazone-d_3. Chromatographic separation was carried out on a Luna C_{18} column (100 mm × 2.0 mm, 3-μm particle size) using an isocratic mobile phase consisting of a 60:40 (v/v) mixture of acetonitrile and 0.1% formic $acid_{(aq)}$. Each sample was run at 0.2 ml/min for a total run time of 2.5 min per sample. Detection and quantification were performed using a mass spectrometer in selected reaction-monitoring mode with positive electrospray ionization at m/z 358.1 → 135.1 for rosiglitazone, m/z 344.2 → 121.1 for N-desmethyl rosiglitazone, m/z 374.1 → 151.1 for *p*-hydroxy rosiglitazone, and m/z 361.1 → 138.1 for rosiglitazone-d_3. The linear ranges of concentration for rosiglitazone, N-desmethyl rosiglitazone, and *p*-hydroxy rosiglitazone were 1–500, 1–150, and 1–25 ng/ml, respectively, with a lower limit of quantification of 1 ng/ml for all analytes. The coefficient of variation for assay precision was less than 14.4%, and the accuracy was 93.3–112.3%. No relevant cross-talk and matrix effect were observed. This method was successfully applied to a pharmacokinetic study after oral administration of a 4-mg rosiglitazone tablet to healthy male Korean volunteers.

1. Introduction

Rosiglitazone (Fig. 1) is a peroxisome proliferator-activated receptor-γ agonist with insulin-sensitizing properties that is commonly prescribed to treat type 2 diabetes mellitus [1,2]. Its bioavailability is approximately 99% after oral dosing in tablet form, and the absorption is rapid, reaching maximal concentration (C_{max}) within 1 h [3]. Rosiglitazone undergoes extensive metabolism with essentially no excretion of the unchanged parent drug in the urine [3]. It is primarily metabolized by CYP2C8, with CYP2C9 contributing to a minor extent [4]. The two major metabolites of rosiglitazone produced by CYP2C8 are *para*-hydroxy rosiglitazone (*p*-OH-R) and N-desmethyl rosiglitazone (N-DmR) (Fig. 1) [4].

Several studies have reported drug interactions with rosiglitazone as a result of inhibition of CYP2C8 (*e.g.*, gemfibrozil and trimethoprim) or CYP2C9 (*e.g.*, fluvoxamine) [5–7]. Additionally, CYP2C8 is genetically polymorphic, with several variants causing functional effects on enzyme activity and expression [8]. Therefore, the simultaneous quantification of rosiglitazone and its major metabolites via CYP2C8/9, N-DmR and *p*-OH-R, in biological fluids is necessary for pharmacokinetic studies of the interactions of CYP2C8-affecting drugs with rosiglitazone.

To detect rosiglitazone in biological samples, several methods using high-performance liquid chromatography (HPLC) with ultraviolet or fluorescence detection have been developed [9–15]. However, these methods require time-consuming and laborious extraction procedures or relatively large sample volumes (~1 ml), as well as lengthy chromatographic run times, limiting their throughput capacity and sensitivity. Liquid chromatography (LC)–tandem mass spectrometry (MS/MS) methods provide rapid and sensitive simultaneous determination of rosiglitazone and N-DmR [7,16], but are only semi-quantitative for N-DmR; due to lack

* Corresponding author at: Department of Clinical Pharmacology and Clinical Trial Center, Inje University Busan Paik Hospital, 633-165, Gaegum-Dong, Jin-Gu, Busan 614-735, South Korea. Tel.: +82 51 890 8969; fax: +82 51 892 1232.
E-mail address: baesk@busanpaik.ac.kr (S.K. Bae).

1570-0232/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.jchromb.2009.05.001

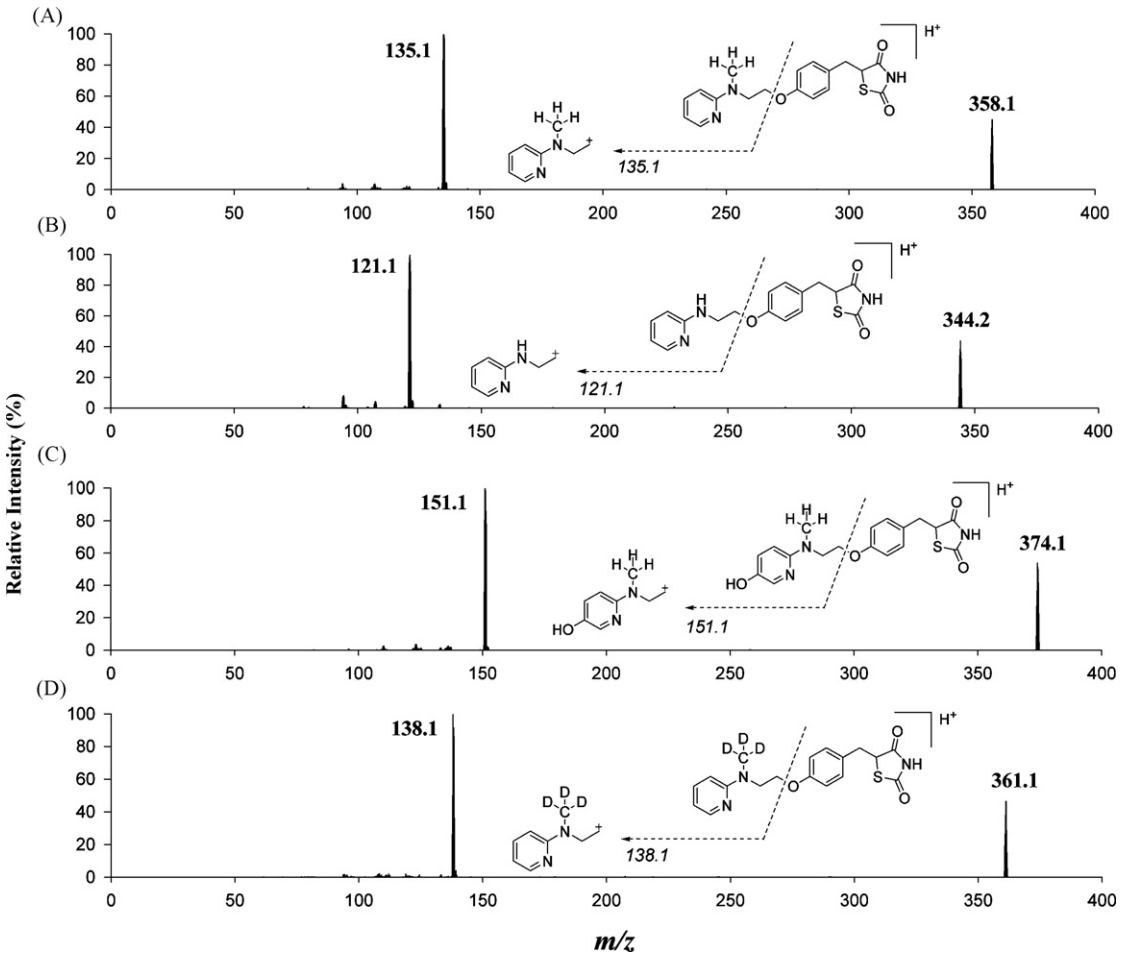

Fig. 1. Product ion mass spectra of rosiglitazone (A), N-desmethyl rosiglitazone (B), *p*-hydroxy rosiglitazone (C), and rosiglitazone-d_3 (IS) (D).

of a reference standard material, plasma concentrations of N-DmR could only be estimated in arbitrary units. Recently, O'Maille et al. [17] reported an LC–MS/MS method that uses liquid–liquid extraction to simultaneously quantify rosiglitazone and N-DmR in human plasma. However, no previous reports have described the simultaneous LC–MS/MS quantification of rosiglitazone, N-DmR, and *p*-OH-R in human plasma.

In the present study, we established a fully validated, accurate, rapid, and sensitive LC–MS/MS method for the simultaneous quantification of rosiglitazone and its two major metabolites via CYP2C8/9, N-DmR and *p*-OH-R, in human plasma. This method, which uses a simple deproteinization procedure, was successfully applied to a pharmacokinetic study of rosiglitazone (Avandia®; 4-mg oral tablet) in six healthy male Korean volunteers.

2. Experimental

2.1. Chemicals

Rosiglitazone maleate, N-DmR, *p*-OH-R, and rosiglitazone-d_3 (R-d_3), which was used as an internal standard (IS), were purchased from Toronto Research Chemicals (North York, ON, Canada) and were at least 98.5% pure. Formic acid was obtained from Sigma–Aldrich (St. Louis, MO, USA). HPLC-grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). All other chemicals and solvents were of the highest analytical grade available. Drug-free heparinized human plasma was obtained from the Clinical Trial Center of Busan Paik Hospital (Busan, South Korea).

2.2. Preparation of standards and quality controls

Stock solutions of rosiglitazone, N-DmR, *p*-OH-R, and R-d_3 were made by dissolving the compounds in acetonitrile at 1 mg/ml. The R-d_3 stock solution was further diluted to 40 ng/ml in acetonitrile for routine use as an IS. For experiments, stock solutions were serially diluted with acetonitrile and added to drug-free plasma to obtain final concentrations of 1, 2, 5, 20, 100, 200, or 500 ng/ml for rosiglitazone; 1, 2, 5, 10, 30, 50, or 150 ng/ml for N-DmR; 1, 2, 4, 5, 10, 15, or 25 ng/ml for *p*-OH-R. On the day of analysis, calibration graphs for rosiglitazone, N-DmR, and *p*-OH-R in human plasma were derived from their peak area ratios relative to that of R-d_3 using linear regression with $1/x$ as a weighting factor. Quality control (QC) samples were assayed along with each batch of plasma samples.

The QC samples were prepared daily by adding 10 μl of diluted stock solution to 90 μl of drug-free human plasma to achieve final concentrations of 1, 3, 50, and 400 ng/ml rosiglitazone; 1, 2, 25, and 100 ng/ml N-DmR; 1, 3, 7.5, and 20 ng/ml *p*-OH-R. The QC samples were used to evaluate the intra- and inter-day precision and accuracy of the method. All prepared plasma samples and stock solutions were stored at −80 °C (Revco ULT 1490 D-N-S; Western Mednics, Asheville, NC, USA).

2.3. Characterization of product ions using tandem mass spectrometry

To characterize the product ions of rosiglitazone, N-DmR, *p*-OH-R, and R-d_3, 1-μM solutions of each compound were separately

infused into the mass spectrometer at 10 μl/min. The precursor ions $[M+H]^+$ and the pattern of fragmentation were monitored in positive ion mode. The major peaks observed in the MS/MS scan were used to quantify rosiglitazone, its two major metabolites, and the IS.

2.4. Analytical system

The concentrations of rosiglitazone, N-DmR, *p*-OH-R, and R-d_3 in human plasma were quantified using LC–MS/MS with an API 3000 LC-MS-MS System (Applied Biosystems, Foster City, CA, USA) equipped with an electrospray ionization interface, used to generate positive ions ($[M+H]^+$). The compounds were separated on a reversed-phase column (Luna C_{18}, 100 mm × 2.0 mm i.d., 3-μm particle size; Phenomenex, Torrance, CA, USA) with an isocratic mobile phase consisting of acetonitrile and 0.1% formic $acid_{(aq)}$ (60:40 (v/v)). The mobile phase was eluted using an Agilent 1100 series pump (Agilent, Wilmington, DE, USA) at 0.2 ml/min.

The turbo ion-spray interface was operated in positive ion mode at 5500 V. The operating conditions, which were optimized by flow injection of a mixture of all analytes, were: nebulizing gas flow, 1.46 l/min; turbo ion-spray gas flow, 8 l/min; curtain gas flow, 1.44 l/min; ring voltage, 200 V; collision gas (nitrogen) pressure, 2.58×10^{-5} Torr. The mass transitions used for rosiglitazone, N-DmR, *p*-OH-R, and R-d_3 were m/z 358.1 → 135.1 (collision energy, 37 eV), 344.2 → 121.1 (31 eV), 374.1 → 151.1 (39 eV), and 361.1 → 138.1 (37 eV), respectively. Quadrupoles Q1 and Q3 were set to unit resolution. The analytical data were processed using Analyst software (Version 1.4.2; Applied Biosystems).

2.5. Sample preparation by deproteinization

A 200-μl aliquot of acetonitrile containing 40 ng/ml R-d_3 was added to a 100-μl aliquot of human plasma sample and mixed by vortexing. After the mixture was centrifuged at 9000 × *g* for 10 min at 4 °C, the supernatant fraction was transferred to another vial. A 20-μl aliquot of the supernatant fraction was injected into the LC–MS/MS system. All prepared samples were kept in an autosampler at 4 °C until injection.

2.6. Method validation

Method validation assays were carried out according to the currently accepted United States Food and Drug Administration's bioanalytical method validation procedures [18]. The validation parameters were selectivity, linearity, sensitivity, accuracy, precision, extraction recovery, matrix effect and stability of rosiglitazone, N-DmR, and *p*-OH-R in human plasma.

Selectivity was evaluated by comparing chromatograms of six different batches of plasma obtained from six subjects to ensure that no interfering peaks were present at the respective retention times of the analytes at the lower limit of quantification (LLOQ) levels. The LLOQ was defined as the lowest concentration of analyte yielding a signal-to-noise ratio of at least 10, acceptable accuracy (80–120%), and sufficient precision (within 20%); LLOQs were verified by the analysis of six replicates.

The cross-talk phenomena among MS/MS channels were assessed injecting rosiglitazone, N-DmR, *p*-OH-R, and IS, separately, at the highest concentrations for calibration curve and monitoring the response in the other MS/MS channels.

The linearity of each method-matched calibration curve was determined by plotting the peak area ratios (y) of rosiglitazone, N-DmR, or *p*-OH-R relative to that of the IS *vs.* the nominal concentration (x) of the same analyte. The calibration curves were constructed by weighted ($1/x$) least squares linear regression.

Intra- and inter-day accuracy and precision for this method were determined at four different concentration levels on 6 different days; on each day, six replicates were analyzed with independently prepared calibration curves. The percentage accuracy was expressed as (mean observed concentration)/(nominal concentration) × 100, and the precision was the relative standard deviation (R.S.D., %).

To evaluate the absolute or relative matrix effect and extraction recovery of rosiglitazone, N-DmR, and *p*-OH-R, six different sources of drug-free plasma were used. Taking the analyte peak areas obtained by direct injection of solvent (or neat) standard solutions as *A*, the corresponding peak areas for solvent (or neat) standard solutions spiked after extraction into plasma extracts as *B*, and peak areas for solvent (or neat) standard solutions spiked before plasma extraction as *C*, the absolute matrix effect and extraction recovery values were calculated as [19]

$$\text{absolute matrix effect } (\%) = \frac{B}{A} \times 100$$

$$\text{extraction recovery } (\%) = \frac{C}{B} \times 100$$

The R.S.D. (%) of the mean peak areas of analytes in the extracted plasma indicated the relative matrix effect. The matrix effect and extraction recovery of the IS were evaluated using the same method. All assays were performed in triplicate at concentrations of 3 and 400 ng/ml for rosiglitazone, 2 and 100 ng/ml for N-DmR, and 3 and 20 ng/ml for *p*-OH-R.

To study analyte stability in human plasma, drug-free plasma samples were spiked with 3 or 400 ng/ml rosiglitazone, 2 or 100 ng/ml N-DmR, or 3 or 20 ng/ml *p*-OH-R. Stability was assessed by analyzing three replicate samples after four different manipulations: (1) short-term storage (24 h at room temperature); (2) long-term storage (90 days at −80 °C); (3) three freeze–thaw cycles; (4) post-treatment storage (8 h at room temperature). The concentrations obtained were compared with the nominal values of the QC samples. The stabilities of the stock solutions of rosiglitazone, N-DmR, and *p*-OH-R, and R-d_3 after 3 weeks at 4 °C and after 4 months at −80 °C were evaluated by comparison with freshly prepared solutions of the same concentrations.

2.7. Clinical application

Six healthy male volunteers who gave written informed consent took part in this study. The exclusion criteria were the presence of health problems, drug or alcohol abuse, or abnormalities in laboratory screening values. The study was approved by the Institutional Review Board of Busan Paik Hospital (Busan, South Korea) and was performed according to the rules of Good Clinical Practice.

After an overnight fast, all subjects were given a oral tablet containing 4 mg of rosiglitazone (Avandia®, GlaxoSmithKline Korea, Seoul, South Korea). Blood samples (~4 ml) were collected *via* a cannula before dosing and 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after dosing. The blood samples were immediately centrifuged at 3000 × *g* for 10 min at 4 °C, and the resulting plasma samples were stored at −80 °C until LC–MS/MS analysis. Pharmacokinetic parameters were calculated by a non-compartmental analysis using WinNonlin Professional software (Version 5.2, Pharsight Corp., Mountain View, CA, USA) using the total area under the plasma concentration–time curve from time zero to infinity ($AUC_{0-\infty}$) or the last measured time (AUC_{0-t}); the logarithmic trapezoidal rule during the declining plasma-level phase [20] and the linear trapezoidal rule for the rising plasma-level phase. The peak plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were read directly from the experimental data.

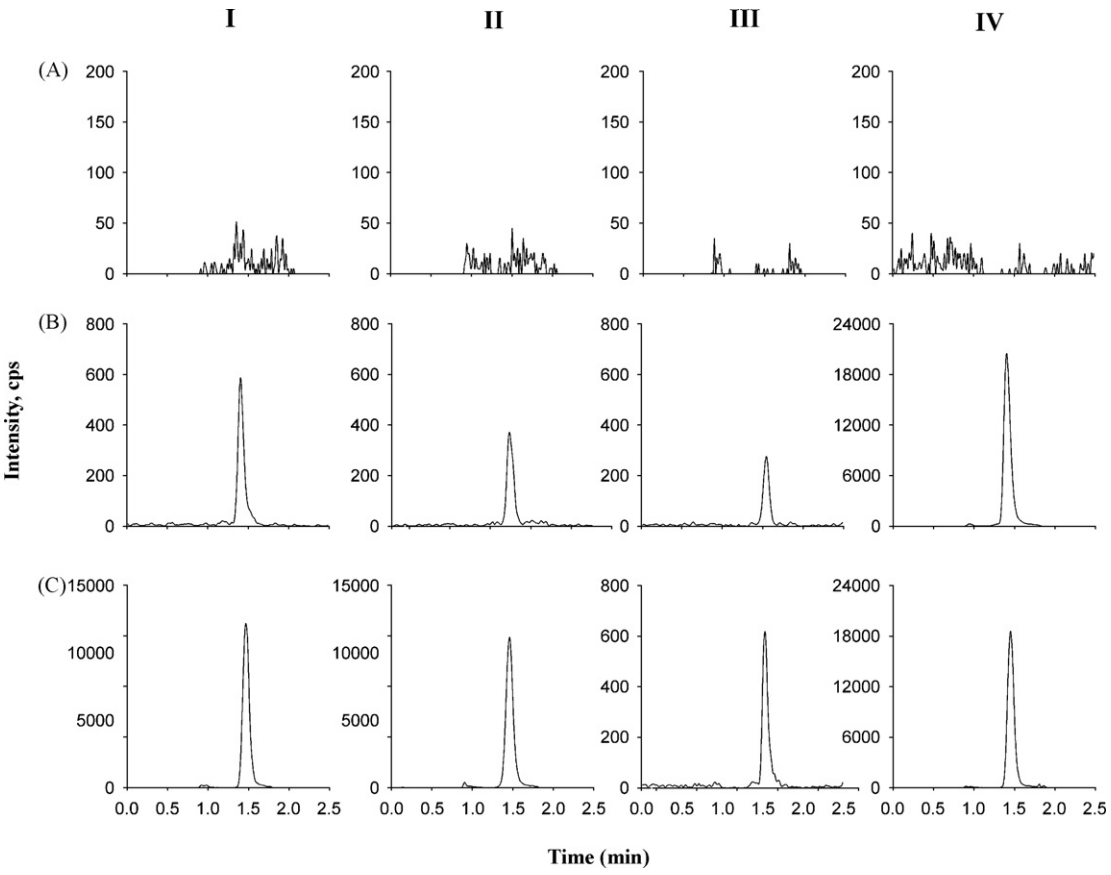

Fig. 2. Representative chromatograms of rosiglitazone (I), N-desmethyl rosiglitazone (II), *p*-hydroxy rosiglitazone (III) and IS (rosiglitazone-d_3) (IV): (A) blank plasma, (B) blank plasma spiked with 2 ng/ml of rosiglitazone, N-desmethyl rosiglitazone, *p*-hydroxy rosiglitazone and IS (40 ng/ml), and (C) a plasma sample obtained from a volunteer 12 h after oral administration of rosiglitazone 4 mg tablet (rosiglitazone: 45.6 ng/ml, N-desmethyl rosiglitazone: 66.5 ng/ml, and *p*-hydroxy rosiglitazone: 4.83 ng/ml).

3. Results and discussion

3.1. Mass spectrometry

In positive ion mode, all analytes yielded protonated molecular ions ($[M+H]^+$) as the major species. The fragmentation patterns of the protonated molecular ions were evaluated by increasing the collision energy. The product ion spectra and fragmentation patterns for rosiglitazone, N-DmR, *p*-OH-R, and R-d_3 are shown in Fig. 1; the greatest intensities were observed at *m*/*z* 135.1, 121.1, 151.1, and 138.1, respectively. The mass parameters were optimized by observing the maximal response of the product ions.

3.2. Method validation

Rosiglitazone, N-DmR, and R-d_3 eluted at 1.5 min, and *p*-OH-R eluted at 1.6 min, respectively. There were no interfering peaks at these elution times and no cross-talk phenomena were observed among MS/MS channels. Typical chromatograms for drug-free plasma, plasma spiked with 2 ng/ml rosiglitazone, N-DmR, and *p*-OH-R, and plasma collected from a volunteer 12 h after oral

Table 1
Intra- and inter-day precision and accuracy data for this assay of rosiglitazone, N-desmethyl rosiglitazone (N-DmR), and *p*-hydroxy rosiglitazone (*p*-OH-R) in human plasma ($n = 6$).

Compounds	Added (ng/ml)	Intra-day			Inter-day		
		Precision		Accuracy (%)	Precision		Accuracy (%)
		Measured (ng/ml)	R.S.D. (%)		Measured (ng/ml)	R.S.D. (%)	
Rosiglitazone	1	1.12 ± 0.0630	5.64	111	1.05 ± 0.0780	7.42	105
	3	3.33 ± 0.0570	1.71	111	3.21 ± 0.254	7.90	105
	50	46.7 ± 3.57	3.57	93.3	48.5 ± 2.69	5.54	96.9
	400	393 ± 2.50	2.50	98.3	408 ± 12.2	2.99	102
N-DmR	1	1.12 ± 0.0510	4.53	112	1.10 ± 0.109	9.83	110
	2	2.17 ± 0.131	6.02	109	2.17 ± 0.0970	4.47	107
	25	25.0 ± 1.08	4.30	100	25.0 ± 1.55	6.18	100
	100	106 ± 1.86	1.75	106	101 ± 5.41	5.36	101
p-OH-R	1	0.970 ± 0.130	13.4	96.9	1.02 ± 0.146	14.4	102
	3	2.88 ± 0.202	7.00	96.1	2.97 ± 0.286	9.61	101
	7.5	7.61 ± 0.709	9.32	101	7.35 ± 0.491	6.68	98.0
	20	21.6 ± 1.28	5.95	108	20.5 ± 1.14	5.54	103

administration of a 4-mg rosiglitazone tablet are shown in Fig. 2. The total run time per sample was 2.5 min.

The calibration curves in human plasma provided reliable responses at rosiglitazone, N-DmR, and *p*-OH-R concentrations of 1–500, 1–150, and 1–25 ng/ml, respectively. The best linear fit and least-squares residuals for the calibration curve were achieved with a weighting factor of $1/x$. During the validation, the mean correlation coefficients (r) in human plasma were 0.9983 (range, 0.9965–0.9999; $n = 6$) for rosiglitazone, 0.9978 (range, 0.9953–0.9993; $n = 6$) for N-DmR, and 0.9973 (range, 0.9945–0.9986; $n = 6$) for *p*-OH-R. The LLOQ for rosiglitazone and its two major metabolites was 1 ng/ml at a signal-to-noise ratio of 10. This sensitivity was sufficient to allow pharmacokinetic studies of rosiglitazone and its two major metabolites after oral rosiglitazone administration.

The intra- and inter-day precision and accuracy of this assay were determined by analyzing six replicates of QC samples at four concentrations on 6 different days (Table 1). The coefficients of variation for the intra- and inter-day precision were <13.4% and <14.4%, respectively. The intra- and inter-day accuracies were 93.3–112% and 96.9–110%, respectively.

The absolute matrix effect percentage (the ratio $B/A \times 100$, as defined in Section 2.6) was between 85 and 115%, indicating no significant matrix effect for any of the analytes. The relative matrix effects in six different lots of plasma were within 15%, indicating that the impact from the extracted plasma matrix was negligible and consistent.

The extraction recoveries of rosiglitazone in plasma were 91.8 and 97.0% at 3 and 400 ng/ml, respectively. For N-DmR, the corresponding values were 91.2 and 98.4% at 2 and 100 ng/ml, respectively, and for *p*-OH-R, they were 90.8 and 98.0% at 3 and 20 ng/ml, respectively. For the IS, the extraction recovery at an initial concentration of 40 ng/ml was 102% (data not shown). The low matrix effect and consistent and highly reproducible recovery of this assay show it to be reliable for routine bioanalysis.

The stock solutions of rosiglitazone, N-DmR, *p*-OH-R, and R-d_3 in acetonitrile were stable for 3 weeks at 4 °C and for 4 months at −80 °C; we obtained more than 97.8 and 98.4% recovery from samples spiked with stock solutions stored under these respective conditions. No significant degradation of any of the analytes in human plasma occurred after short-term storage for 24 h at room temperature, long-term storage for 90 days at −80 °C, three freeze–thaw cycles, or post-treatment storage for 8 h at room temperature, with ±15% deviation between the predicted and nominal concentrations (data not shown).

3.3. Clinical application

The LC–MS/MS method described herein was successfully applied to a pharmacokinetic study of rosiglitazone. The mean plasma concentration–time profiles for rosiglitazone, N-DmR, and *p*-OH-R in six healthy male Korean volunteers after oral administration of a 4-mg rosiglitazone tablet are shown in Fig. 3, and some relevant pharmacokinetic parameters are summarized in Table 2. The sensitivity and specificity of the method were sufficient for characterizing the pharmacokinetics of rosiglitazone and its two major metabolites. The QC samples ranged within 15% of the nominal concentrations, meeting the acceptance criteria of the United States Food and Drug Administration for the validation of bioanalytical methods [18]. The mean C_{max} of rosiglitazone was 340 ± 68.3 ng/ml occurring at T_{max} (0.67 h; range, 0.33–1 h). The terminal half-life and $AUC_{0-\infty}$ values of rosiglitazone were 4.33 ± 1.07 h and 1970 ± 554 ng h/ml, respectively. The mean C_{max} of N-DmR was 114 ± 20.3 ng/ml at T_{max} (6 h; range, 4–8 h), and the AUC_{0-t} values were 2010 ± 327 ng h/ml. The pharmacokinetic parameters of rosiglitazone and N-DmR were similar to those reported in the literature [5–7,16,17]. Although the plasma concentration of *p*-OH-R was low compared with that of N-DmR, *p*-OH-R was detectable and sufficiently measurable at all sampling points except for the first sampling time, 0.33 h (Fig. 3). As described earlier, to date there is no information regarding the pharmacokinetic properties of *p*-OH-R, one of the major metabolites of rosiglitazone via CYP2C8/9, after rosiglitazone administration. Additionally, it was reported [21] that the sulphate conjugate of *p*-OH-R, *para*-O-

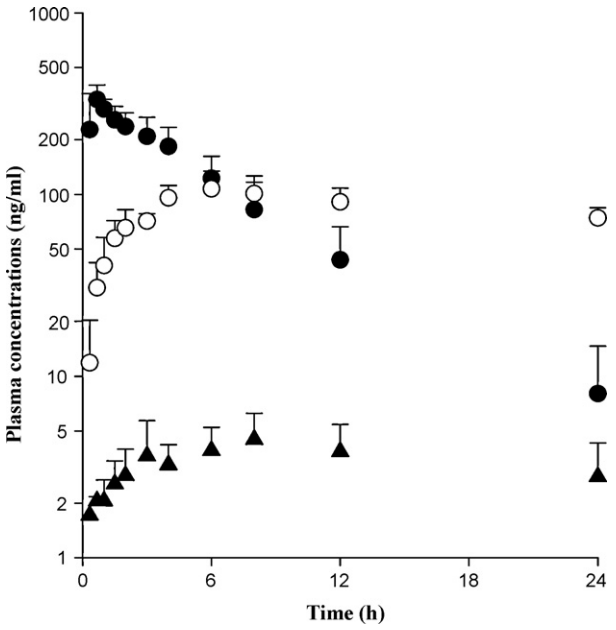

Fig. 3. Plasma concentration–time profiles for rosiglitazone (●), N-desmethyl rosiglitazone (○), and *p*-hydroxy rosiglitazone (▲) after oral administration of a rosiglitazone 4 mg tablet to six Korean healthy male volunteers. Vertical bars represent standard deviation.

Table 2
Pharmacokinetic parameters of rosiglitazone, N-desmethyl rosiglitazone (N-DmR), and *p*-hydroxy rosiglitazone (*p*-OH-R) after single oral administration rosiglitazone 4 mg tablet to six Korean healthy male volunteers.

Parameters[a]	Rosiglitazone	N-DmR	*p*-OH-R
AUC_{0-t} (ng h/ml)[b]	1910 ± 506	2010 ± 327	81.9 ± 27.5
$AUC_{0-\infty}$ (ng h/ml)	1970 ± 554		
$t1/2$ (h)	4.33 ± 1.07		
CL/F (l/h/kg)[c]	0.0306 ± 0.00970		
C_{max} (ng/ml)	340 ± 68.3	114 ± 20.3	5.66 ± 1.71
T_{max} (h)[d]	0.67 (0.33–1)	6 (4–8)	8 (3–12)

[a] Values are mean ± standard deviation.
[b] The area under the plasma concentration–time curve from time zero to last sampling time.
[c] Oral clearance of rosiglitazone (clearance over oral bioavailability).
[d] Median (ranges).

sulphate rosiglitazone was the major metabolite in human plasma after oral and intravenous administration of ^{14}C-rosiglitazone. Based on this information, plasma concentrations of unconjugated *p*-OH-R and N-DmR might be influenced by variability of sulfation or glucuronidation activities due to genetic polymorphisms and environmental factors in the absence of any CYP2C8/9-mediated interactions.

4. Conclusions

This report describes the development and validation of a method for the simultaneous quantification of rosiglitazone and its two major metabolites via CYP2C8/9, N-DmR and *p*-OH-R, in human plasma by LC–MS/MS. The method is rapid (2.5 min per sample), sensitive (LLOQ, 1 ng/ml for all analytes), and simple (protein precipitation with acetonitrile). It was also successfully applied to a pharmacokinetic study of rosiglitazone. This method could be used to investigate the mechanisms of CYP2C8/2C9-mediated drug interactions or CYP2C8/2C9 genetic polymorphism of rosiglitazone in clinical trials.

Acknowledgement

This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MOEST) (No. R13-2007-023-00000-0).

References

[1] H. Yki-Järvinen, N. Engl. J. Med. 351 (2004) 1106.
[2] J.A. Balfour, G.L. Plosker, Drugs 57 (1999) 921.
[3] 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.
[4] S.J. Baldwin, S.E. Clarke, R.J. Chenery, Br. J. Clin. Pharmacol. 48 (1999) 424.
[5] M. Niemi, J.T. Backman, M. Granfors, J. Laitila, M. Neuvonen, P.J. Neuvonen, Diabetologia 46 (2003) 1319.
[6] 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.
[7] R.S. Pedersen, P. Damkier, K. Brosen, Br. J. Clin. Pharmacol. 62 (2006) 682.
[8] D. Dai, D.C. Aeldin, J.A. Blaisdell, B. Chanas, S.J. Coulter, B.I. Ghanayem, J.A. Goldstein, Pharmacogenetics 11 (2001) 597.
[9] B.L. Kolte, B.B. Raut, A.A. Deo, M.A. Bagool, D.B. Shinde, J. Chromatogr. B 788 (2003) 37.
[10] A.M. Muxlow, S. Fowles, P. Russell, J. Chromatogr. B 752 (2001) 77.
[11] R.N. Mamidi, M.R. Chaluvadi, B. Benjamin, M. Ramesh, K. Katneni, A.P. Babu, J. Bhanduri, N.M. Rao, R. Rajagopalan, Arzneimittelforschung 52 (2002) 560.
[12] T. Radhakrishna, J. Satyanarayana, A. Satyanarayana, J. Pharm. Biomed. Anal. 29 (2002) 873.
[13] M.W. Hrusak, R.F. Frye, J. Chromatogr. B 803 (2004) 317.
[14] R.S. Pedersen, K. Brosen, F. Nielsen, Chromatographia 62 (2005) 197.
[15] K.A. Kim, J.Y. Park, Biomed. Chromatogr. 18 (2004) 613.
[16] 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.
[17] G. O'Maille, S.M. Pai, X. Tao, G.T. Douglas Jr., R.G. Jenkins, J. Pharm. Biomed. Anal. 48 (2008) 934.
[18] US FDA, Guidance for Industry/Bioanalytical Method Validation, US Food and Drug Administration, Rockville, MD, 2001.
[19] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019.
[20] W.L. Chiou, J. Pharmacokinet. Biopharm. 6 (1978) 539.
[21] P.J. Cox, D.A. Ryan, F.J. Hollis, A.-M. Harris, A.K. Miller, M. Vousden, H. Cowley, Drug Metab. Dispos. 28 (2000) 772.