Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 803 (2004) 317–320
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Simplified method for determination of rosiglitazone in human plasma

Matthew W. Hruska, Reginald F. Frye*

Department of Pharmaceutical Sciences, Pharmacodynamic Research Center, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15261, USA

Received 4 September 2003; received in revised form 8 January 2004; accepted 8 January 2004

Abstract

Rosiglitazone is a thiazolidinedione antihyperglycemic drug used in the treatment of type 2 diabetes mellitus. Rosiglitazone is extensively metabolized by cytochrome P450 2C8 and so may have some utility as an in vivo probe for this enzyme. A liquid chromatographic method using sensitive fluorescence detection and simplified sample processing involving protein precipitation with acetonitrile was developed. The isocratic mobile phase consisted of 10 mM sodium acetate–acetonitrile (pH 5; 60:40, v/v) and was delivered at a flow rate of 1 ml/min to an Alltima phenyl column (250 mm × 4.6 mm, 5 microm). Detection was by fluorescence at (EX/EM) 247/367 for rosiglitazone and 235/310 for the internal standard betaxolol. Intra- and inter-day precision ranged from 3.1 to 8.5% and 2.3 to 5.7%, respectively. No endogenous interference was observed with either rosiglitazone or the internal standard. The assay is simple, economical, precise, and is directly applicable to human pharmacokinetic studies involving single dose rosiglitazone administration.

Keywords: Rosiglitazone

1. Introduction

Rosiglitazone (Fig. 1) is a thiazolidinedione antihyperglycemic drug used in the treatment of type 2 diabetes mellitus. Thiazolidinedione antihyperglycemic agents exert their effect through the peroxisome proliferator-activated receptor-γ (PPAR-γ), which facilitates the expression of genes responsible for glucose and lipid metabolism [1]. Rosiglitazone has a half-life in humans of approximately 3–4 h, a volume of distribution of approximately 18 l, and it is 99.8% bound to plasma proteins [1]. Rosiglitazone undergoes extensive metabolism with essentially no parent drug excreted unchanged in the urine. The two major metabolites of rosiglitazone isolated via microsomal and animal studies are *para*-hydroxy rosiglitazone and *N*-desmethyl rosiglitazone, both of which are produced by cytochrome P450 (CYP) 2C8 [2,3].

Since rosiglitazone is predominantly metabolized by CYP2C8, it may have potential use as an in vivo probe of this enzyme. Availability of a simple method for determination of rosiglitazone in human plasma would aid in the evaluation of rosiglitazone as a CYP2C8 probe. Several methods have been published but each has drawbacks limiting ease of use. Muxlow et al., developed a method using the automated sequential trace enrichment of dialysates (ASTED) system combined with fluorescence detection [4]. A drawback of this method is that it requires a specialized extraction system (ASTED) and it uses a synthesized internal standard, both of which are not readily available. Also, the linear standard curve range extended only to 100 ng/ml, which is not ideal for human pharmacokinetic studies of rosiglitazone. After a single dose of rosiglitazone, maximum plasma concentrations exceed 600 ng/ml [5]. Mamidi et al., reported a method that is linear up to 1000 ng/ml, but uses an internal standard that is not commercially available [6]. The method of Kolte et al., utilizes HPLC linked to ultraviolet detection and a commercially available compound (pioglitazone) as the internal standard [7]. However, the method requires a large sample volume (1 ml) and a prolonged analysis time of 25 min per sample.

* Corresponding author. Present address: College of Pharmacy, University of Florida, P.O. Box 100486, Gainesville, FL 32610, USA. Tel.: +1-352-392-8551; fax: +1-352-392-9388.
E-mail address: frye@cop.ufl.edu (R.F. Frye).

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2004.01.010

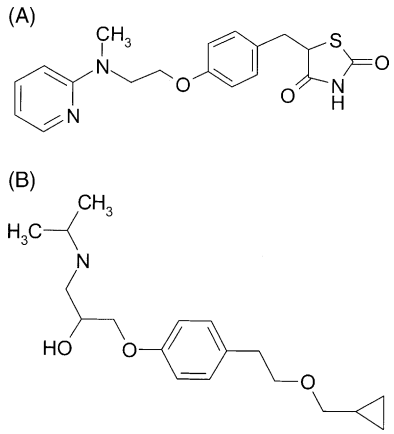

Fig. 1. Structures of (A) rosiglitazone and (B) betaxolol (internal standard).

Thus, the goal of this work was to establish a method that has a simple and inexpensive extraction method, requires a small plasma sample volume, and has a readily available internal standard. In this paper, we introduce an HPLC fluorescence method with simplified sample processing to quantify rosiglitazone in human plasma. The method was used to determine rosiglitazone plasma concentrations after single-dose administration.

2. Experimental

2.1. Chemicals and reagents

Rosiglitazone reference standard (≥98%) was purchased from Cayman Chemical Company (Ann Arbor, MI, USA). Betaxolol reference standard (100%) was obtained from United States Pharmacopeia (Rockville, MD, USA). Sodium acetate and acetonitrile (ACN) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Blank human plasma was purchased from the Central Blood Bank of Pittsburgh (Pittsburgh, PA, USA). HPLC grade deionized water was obtained from an in-house Millipore (Billerica, MA, USA) water system.

2.2. Instrumentation and chromatographic conditions

The HPLC system consisted of a Waters 501 Pump coupled with a Waters model 2475 fluorescence detector (Waters Corp., Milford, MA). Detection and quantification was performed using Millennium^{32} software version 3.1 (Waters Corp., Milford, MA). Separation was achieved with a 250 mm × 4.6 mm, 5 μm Alltima phenyl column (Alltech Associates Inc., Deerfield, IL) and an isocratic mobile phase of 10 mM sodium acetate (pH 5)–ACN (60:40, v/v) delivered at a flow rate of 1 ml/min. Rosiglitazone was monitored at λ_{ex} of 247, λ_{em} of 367 and betaxolol was monitored at λ_{ex} of 235 and λ_{em} of 310. During each sample run, λ_{em} = 247 and λ_{em} = 367 was monitored from 0 to 7 and 10 to 15 min, and λ_{ex} = 235 and λ_{em} = 310 was monitored from 7 to 10 min. The total run time was 15 min.

2.3. Standard preparation

Rosiglitazone stock solution was prepared at a concentration of 1 mg/ml in ethanol. Dilutions that were prepared in ethanol at concentrations of 200 ng/ml, and 2, 10, and 20 μg/ml were used to prepare calibration standards and quality control (QC) samples. The internal standard (IS) betaxolol was dissolved in methanol to prepare a 25 μg/ml stock solution. Blank plasma was spiked with appropriate stock solutions and brought up to 200 μl prior to processing.

2.4. Sample preparation

IS (10 μl or 250 ng) was added to plasma samples (200 μl) in microcentrifuge tubes and vortexed briefly. ACN (600 μl) was then added to each sample, vortexed for 2 min, and centrifuged at 3000 × *g* for 10 min. Supernatant was evaporated using nitrogen gas in a heating block set at 45 °C. Dried samples were reconstituted with 200 μl of mobile phase and a 75 μl aliquot was injected onto the column.

2.5. Calibration and linearity

Calibration curves consisted of seven standard concentrations of rosiglitazone in human plasma: 5, 10, 50, 100, 250, 500, and 1000 ng/ml. Duplicate calibration curves were analyzed daily for 3 days, with the lowest concentration (5 ng/ml) prepared in triplicate. For each curve, the rosiglitazone peak height to IS peak height ratio was calculated and plotted against nominal rosiglitazone concentrations. Calibration curves for rosiglitazone were constructed by weighted ($1/y^2$) linear regression analysis.

2.6. Precision and accuracy

Precision and accuracy were determined by the analysis of rosiglitazone QC samples spiked at concentrations of 40, 400, and 750 ng/ml. Replicate QC samples (n = 12) were analyzed on day 1 to determine intra-day precision and accuracy. Inter-day precision and accuracy were determined by replicate QC samples on day 1 (n = 12), day 2 (n = 6), and day 3 (n = 6), for a total of n = 24 QC samples at each concentration. Mean, standard deviation, and relative standard deviation (R.S.D.) were calculated from QC values and used in the estimation of intra- and inter-day precision. Accuracy (bias) is expressed as the percent difference between the calculated mean concentration relative to the nominal concentration.

2.7. Selectivity and stability

Selectivity was assessed by processing six separate sources of blank plasma. Blank plasma samples were

processed in duplicate and compared to plasma spiked with the lowest rosiglitazone standard. Sample carryover was determined by inserting vials of blank mobile phase in random positions throughout the third validation. Batches of high and low QCs were prepared and subjected to three freeze–thaw cycles (−80 °C to room temperature) prior to processing and analysis. After each freeze thaw cycle, aliquots were extracted and analyzed. To determine stability of processed samples, high and low QC samples were prepared and subjected to repeated analysis over a 24 h period post-extraction.

2.8. Extraction efficiency

Extraction efficiency was determined by comparing the response obtained from extracted QC samples versus the response observed after direct injection of reference samples. Reference samples consisted of water spiked with appropriate amounts of rosiglitazone. Responses obtained from reference samples were defined as 100%.

2.9. Application to plasma sampling

Rosiglitazone pharmacokinetics were evaluated in a healthy volunteer. The protocol was approved by the University of Pittsburgh Institutional Review Board and signed informed consent was obtained. Rosiglitazone 8 mg (Avandia®, GlaxoSmithKline, Philadelphia, PA, USA) was administered orally with 8 oz of water after an overnight fast. Plasma was collected for 24 h, and stored at −20 °C until analyzed. Plasma concentrations of rosiglitazone were determined as described above. The maximum observed rosiglitazone concentration (C_{max}) and the time at which C_{max} was observed (T_{max}) are reported. The terminal elimination rate constant (λz) was estimated by nonlinear least squares regression analysis of the terminal portion of the concentration–time curve. The apparent elimination half-life was calculated as $0.693/\lambda z$.

3. Results

3.1. Chromatography

Representative chromatograms of plasma samples are depicted in Fig. 2. Fig. 2A depicts a blank plasma sample and a plasma sample spiked with rosiglitazone 5 ng/ml (LOQ); Fig. 2B shows a plasma sample obtained 1 h after a single dose of rosiglitazone 8 mg. Retention times were approximately 7.9 and 13.2 min for IS and rosiglitazone, respectively. The peaks of interest were well separated and free from interference with endogenous substances.

3.2. Precision, linearity, and accuracy

Linear calibration curves were obtained for rosiglitazone over the concentration range of 5–1000 ng/ml; the mean regression equation was: $Y = [0.0058 \pm 0.0020]X + [0.0022 + 0.0018]$. The correlation coefficients calculated for each run were >0.99. Intra- and inter-day precision (R.S.D.) was ≤8.5% and accuracy was within ±11.1% (Table 1). The signal to noise ratios for rosiglitazone at 5 ng/ml and the IS at 250 ng were greater than 5:1 (Fig. 2A).

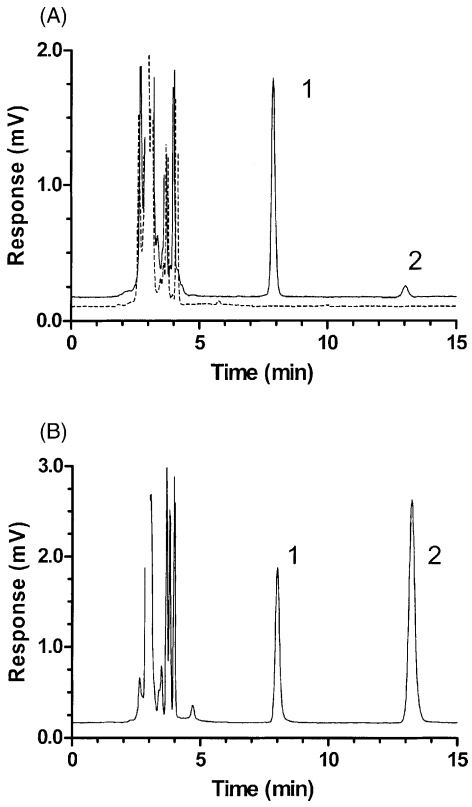

Fig. 2. Representative chromatographs of (A) extracted blank plasma (- - -) and plasma spiked (—) at LOQ concentration of rosiglitazone 5 ng/ml (offset = 0.05 mV); (B) rosiglitazone in plasma at 1 h after single dose rosiglitazone 8 mg administration (concentration 210.2 ng/ml). Peaks: (1) IS, (2) rosiglitazone.

3.3. Selectivity, stability, and recovery

No endogenous interference with rosiglitazone or IS was observed in six different sources of blank plasma. There was no evidence of sample carryover. Rosiglitazone and IS were both stable in the injection solvent at room temperature for at least 24 h prior to injection. Samples subjected to three freeze–thaw cycles showed no degradation of rosiglitazone. Extraction recovery determined at the three QC concentrations was ∼100% (Table 2).

3.4. Plasma sampling

The log concentration–time profile for a healthy subject administered rosiglitazone 8 mg as a single dose is illustrated in Fig. 3. The observed C_{max} was 697.3 ng/ml, which occurred at a T_{max} of 0.5 h; the observed $t_{1/2}$ was 3.9 h.

Table 1
Intra- and inter-day precision and accuracy of rosiglitazone in plasma

Concentration (ng/ml)		R.S.D. (%)	Bias (%)
Spiked	Observed (mean ± S.D.)		
Intra-assay precision ($n = 12$)			
Quality controls			
40	38.5 ± 12	3.1	−3.8
400	367.2 ± 18.9	5.1	−8.2
750	759.6 ± 64.6	8.5	1.3
Inter-assay precision ($n = 24$)			
Quality controls			
40	38.1 ± 0.9	2.3	−4.8
400	355.8 ± 16.1	4.5	−11.1
750	706.0 ± 40.5	5.7	−5.9
Standards			
5	5.1 ± 0.3	6.0	1.1
10	10.0 ± 0.6	5.8	−0.4
50	50.5 ± 2.5	4.9	1.0
100	98.7 ± 3.9	4.0	−1.3
250	246.4 ± 5.6	2.3	−1.5
500	531.6 ± 43.8	8.2	6.3
1000	982.1 ± 28.2	2.9	−1.8

Table 2
Extraction recovery of rosiglitazone from human plasma ($n = 3$)

Spiked concentration (ng/ml)	Recovery (%)	R.S.D. (%)
40	105.7 ± 5	4.68
400	113.8 ± 2	1.36
750	105.6 ± 1	0.94

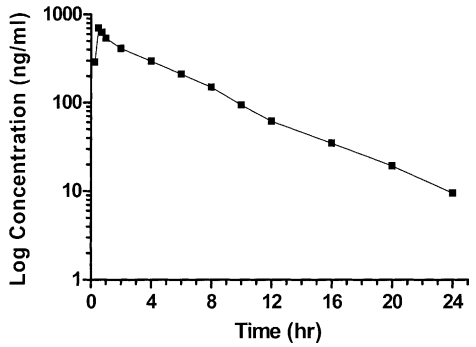

Fig. 3. The log concentration–time profile for subject administered single dose rosiglitazone (8 mg).

4. Discussion

The method presented here allows for simple and economical detection of rosiglitazone in small volumes of human plasma, using protein precipitation coupled with sensitive fluorescent detection. The extraction technique does not require additional expensive solid phase extraction equipment such as the ASTED system [4,5]. Further, this method uses a commercially available fluorescent compound as an internal standard, rather than a synthesized thiazolidinedione-like structure as an internal standard [5,6]. Although the method developed by Muxlow et al. [4], was utilized in the clinical development of rosiglitazone, the standard range had a maximum concentration of 100 ng/ml. Single dose rosiglitazone yields maximum plasma concentrations well above a threshold of 100 ng/ml, as shown by the data in Fig. 3 and other studies [5,6], so an additional dilution step would be required to fit within this standard curve range. A more recent method described by Kolte et al. [7], used a commercially available product as an internal standard but required a much larger volume of plasma due to less sensitive ultraviolet detection. In the method presented here, smaller volumes of plasma can be analyzed since we used fluorescence detection, which has greater sensitivity than ultraviolet detection. By extracting smaller plasma samples, less organic solvents may be used in extraction, yielding less cost and less time drying extracted samples. The analysis time is also much shorter in this assay, 15 min versus 25 min, which cuts down on overall time of sample analysis. Since there is no endogenous interferences in the regions where rosiglitazone and the IS elute (Fig. 2), the analysis time could be decreased even further by using a shorter column.

In this paper, we have introduced a simplified HPLC fluorescence method to quantify rosiglitazone in human plasma. The method provides an economical, simple alternative to the methods previously published and is suitable for the determination of rosiglitazone plasma concentrations after single-dose administration.

Acknowledgements

This project was supported in part by NIH Research Grant R01 MH63458, funded by the National Institute of Mental Health and the Office of Dietary Supplements and NIH/NCRR/GCRC#5M01RR00056.

References

[1] J.A. Balfour, G.L. Plosker, Drugs 57 (1999) 921.
[2] G.C. Bolton, J.P. Keogh, P.D. East, F.J. Hollis, A.D. Shore, Xenobiotica 26 (1996) 627.
[3] S.J. Baldwin, S.E. Clarke, R.J. Chenery, Br. J. Clin. Pharmacol. 48 (1999) 424.
[4] A.M. Muxlow, S. Fowles, P. Russell, J. Chromatogr. B 752 (2001) 77.
[5] 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.
[6] 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.
[7] B.L. Kolte, B.B. Raut, A.A. Deo, M.A. Bago, D.B. Shinde, J. Chromatogr. B 788 (2003) 37.