Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Journal of Chromatography B, 826 (2005) 220–225
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Bioanalysis and pharmacokinetics of the p38 MAPkinase inhibitor SB202190 in rats

Jai Prakash*, Vinay Saluja, Jan Visser, Frits Moolenaar, Dirk K.F. Meijer, Klaas Poelstra, Robbert J. Kok

Department of Pharmacokinetics and Drug Delivery, Groningen University Institute for Drug Exploration (GUIDE), University of Groningen, Antonius Deusinglaan 1, 9713 AV, Groningen, The Netherlands

Received 3 May 2005; accepted 2 September 2005
Available online 23 September 2005

Abstract

We have developed a sensitive and reproducible high performance liquid chromatography (HPLC)-UV method for the quantification of the p38 MAPkinase inhibitor SB202190 in serum, kidney homogenates and urine samples. Liquid–liquid extraction of SB202190 from the samples was performed using diethylether after adding a derivative of SB202190 as internal standard (I.S.). Chromatography was carried out using a C8 reversed-phase column with an isocratic mobile phase consisting of acetonitrile–water–trifluoroacetic acid (30:70:0.1, v/v/v; pH 2.0). Both drug and I.S. were measured at 350 nm and eluted at 5.0 and 10.6 min, respectively. Peak-height ratios of the drug and the I.S. were used for the quantification of SB202190 from the different matrixes. The limit of quantitation of SB202190 in serum, kidney and urine were 0.25 μg/ml, 1 μg/g and 1 μg/ml, respectively. The average recoveries were 74, 75 and 92% in serum, kidney and urine, respectively. The intra- and inter-day precision (% CV) and accuracy (% bias) were below 15% for all concentrations. The method was successfully applied for a pharmacokinetic study of SB202190 in rats.
© 2005 Elsevier B.V. All rights reserved.

Keywords: Liquid–liquid extraction; High performance liquid chromatography; Pharmacokinetic studies; Drug analysis; Renal fibrosis

1. Introduction

In recent years, p38 mitogen activated protein kinase (p38 MAPK) has been investigated extensively and found to play a pivotal role in the etiology of various inflammatory diseases such as septic shock, arthritis and other joint diseases [1–3]. Several p38 MAPK inhibitors have been developed and are currently being tested as anti-inflammatory therapeutics [3]. In addition, the use of these inhibitors has recently been proposed for the treatment of end-stage organ diseases such as renal fibrosis [4].

SB202190 (4-(4-fluorophenyl)-2-(4-hydroxyphenyl)-5-(4-pyridyl)1H-imidazole, Fig. 1) is a well known selective p38 MAPK inhibitor which is often used in various studies to display the role of the p38 MAPK pathway [5–8]. We have recently developed a renal-specific delivery formulation of SB202190 to treat renal fibrosis. To study the pharmacokinetics of SB202190 in different biological samples, we now report a sensitive, accurate and reproducible method. A C_8 reversed-phase column was used for chromatographic separations and the drug was detected specifically by UV measurement at 350 nm. Since the drug levels can be quite low and variable in different samples, we used a liquid–liquid extraction method to isolate the drug from serum, kidney homogenate and urine samples. In order to correct for variation in the extraction, we used a pyridinyl imidazole compound that has similar chemical properties as SB202190 as internal standard (I.S.). We applied the method for the pharmacokinetic analysis of SB202190 in rats after intravenous administration.

2. Experimental

2.1. Materials

SB202190 was purchased from L.C. Laboratories (Woburn, MA, USA) and the purity was at least 99%. The pyridinyl imidazole compound that was used as internal standard (VI7004D) was kindly gifted by Professor G. Keri (Vichem Chemie, Budapest, Hungary). Acetonitrile (HPLC grade) was purchased

* Corresponding author. Tel.: +31 50 3633281; fax: +31 50 3633247.
E-mail address: J.Prakash@rug.nl (J. Prakash).

1570-0232/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2005.09.005

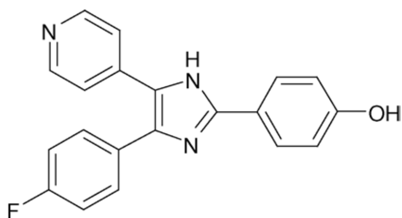

Fig. 1. Chemical structure of SB202190.

from Biosolve, Valkenswaard, The Netherlands. Diethylether was purchased from Boom B.V., Meppel, The Netherlands. All other chemicals were of analytical-reagent grade. Water was purified to ultra-pure water of 18.2 MΩ resistance by Arium 611 (Sartorius AG, Goettingen, Germany).

Drug-free serum, urine and kidneys were obtained from healthy male Wistar rats (250–300 g) provided by Harlan (The Netherlands).

2.2. Instrumental

HPLC analyses were performed using a chromatographic system consisting of a Waters 510 HPLC pump, a column oven set at 40 °C and a Waters 715 Ultra WISP autoinjector (Waters Inc., Milford, MA, USA) in combination with a Spectroflow 757 UV detector (Kratos, Kanagawa, Japan). Separations were achieved with a C_8 reversed-phase column (250 mm × 4.6 mm i.d., 5 μm particle size) purchased from Thermo-Hypersil Keystone (Bellefonte, PA, USA), preceded by a C_{18} GuardPak precolumn (Waters). The mobile phase consisted of acetonitrile–water–trifluoroacetic acid (30:70:0.1, v/v/v; pH 2.0) at a flow rate of 1 ml/min. The effluents were monitored at 350 nm.

2.3. Preparation of stocks, calibration standards and quality control samples

The standard solutions of SB202190 and internal standard (100 μg/ml) were prepared in methanol and kept at 4 °C with light protection. Fresh working standards (10–20 μg/ml) were prepared by diluting the main standards in water.

Calibrations standards were prepared by adding different concentrations of the working standards in drug-free serum, kidney homogenate and urine. Serum and kidney homogenate standards were prepared at concentrations of 0.25, 0.5, 1, 2, 3 and 5 μg/ml and 1, 2, 4, 8, 12 and 20 μg/g of tissue, respectively. Urine calibration standards were prepared at concentrations of 1, 2.5, 5, 10, 15 and 20 μg/ml. Four pools of quality control (QC) samples were prepared by spiking the biological matrixes at the concentrations of 0.25, 1, 2 and 5 μg/ml (serum) 1, 4, 8 and 20 μg/g (kidney homogenate) and 1, 5, 10, and 20 μg/ml (urine).

2.4. Sample preparations

2.4.1. Analysis of SB202190 in serum samples

A 100 μl aliquot of serum was spiked with 25 μl of the I.S. solution in water (20 μg/ml) in a glass tube and vortexed for 30 s. Subsequently, 2 ml of diethylether was added and the sample was vortexed for another 2 min. The mixture was centrifuged at 900 × *g* for 5 min at ambient temperature and the aqueous layer was frozen in liquid nitrogen. The upper organic layer was transferred to another borosilicate glass tube and evaporated completely at 50 °C. The extraction procedure was repeated twice and the total residue was reconstituted in 100 μl of mobile phase. A 25 μl of the reconstituted sample was injected into the HPLC system. The sensitivity of the detector was 0.02 AUFS.

2.4.2. Analysis of SB202190 in kidney homogenate

Kidney homogenate was prepared in 0.1 M phosphate buffer saline (pH 7.2) in 1:3 (w/v) using an ultra-turrax-T25 apparatus (20,000 rpm, 1 min). To 200 μl of homogenate, 50 μl of the I.S. solution in water (20 μg/ml) was added in a glass tube and vortexed for 30 s. The extraction was performed by adding 2 ml of diethylether and vortexing for 2 min. The mixture was centrifuged at 900 × *g* for 5 min and the aqueous layer was frozen in liquid nitrogen. The upper organic layer was transferred to another borosilicate glass tube and evaporated completely at 50 °C. The extraction procedure was repeated twice and the total residue was reconstituted in 200 μl of mobile phase. A 25 μl of the reconstituted sample was injected into the HPLC system. The kidney homogenate samples were estimated at the sensitivity of 0.02 AUFS.

2.4.3. Analysis of SB202190 in urine

One hundred microliters of urine was spiked with 100 μl of the I.S. solution in phosphate buffer saline (20 μg/ml) in a glass tube and vortexed for 30 s. Subsequently, 2 ml of diethylether was added and the sample was vortexed for 2 min. The mixture was centrifuged at 900 × *g* for 5 min at ambient temperature and the aqueous layer was frozen in liquid nitrogen. The upper organic layer was transferred to another borosilicate glass tube and evaporated completely at 50 °C. The extraction procedure was repeated twice and the total residue was reconstituted in 100 μl of mobile phase. A 25 μl of the reconstituted sample was injected into the HPLC system. Urine samples were estimated at 0.1 AUFS.

2.5. Assay validation

Validation of this method was performed in compliance with the current FDA guidelines for biological method validation [9].

2.5.1. Quantifications

To quantify the concentration of SB202190 in QC or unknown samples, peak-height ratios of SB202190 to that of the I.S. were calculated and related to standard curves of SB202190 in drug-free matrix. Lower limits of detection (LLOD) and quantitation (LLOQ) were determined from signal-to-noise ratios. The LLOD is defined as the lowest concentration level resulting to a signal-to-noise ratio of 3:1, while the signal-to-noise ratio should be 10:1 for the LLOQ.

2.5.2. Recovery

The recoveries of SB202190 from serum, kidney homogenate and urine were determined for different standard concentrations by spiking the drug into the corresponding blank sample. The percentage recovery was calculated by comparing the peak-heights of extracted samples with samples in which the compound was spiked directly in mobile phase. Recoveries at four QC concentration levels for serum, kidney homogenate and urine were examined at least five times for each sample type.

2.5.3. Linearity, precision and accuracy

For the construction of calibration curves in serum, kidney homogenate and urine, six different calibration standards of SB202190 were prepared and processed as mentioned above. Six different calibration curves were constructed in serum and kidney homogenate and four different calibration curves in urine. To evaluate precision, at least five QC samples of four different concentrations, to represent the entire range of the standard curve, from each type of biological matrix were processed and injected on a single day (intra-day) and at different days (inter-day). The variability was expressed as the coefficient of variation (% CV) which should be ≤15% at all the concentrations except lower-limit of quantitation (LLOQ) whose value should not be more than 20%. Accuracy of the method is established from the same QC samples. Accuracy is expressed as % bias which should be within limits of ±15% at all concentrations.

2.6. Stability studies

The stabilities of SB202190 and I.S. standards (5 μg/ml in methanol) at room temperature were determined by placing the solutions at the bench up to 24 h.

To evaluate long-term stability of SB202190 in biological samples, at least three QC samples of serum (spiked with SB202190 at 0.25 and 5 μg/ml), kidney homogenate (1 and 20 μg/g) and urine (1 and 20 μg/ml) were stored at −80 °C for 3 weeks.

Freeze–thaw stability of SB202190 in biological samples was determined by subjecting QC samples of serum (0.25 and 5 μg/ml), kidney homogenate (1 and 20 μg/g) and urine (1 and 20 μg/ml) to three freeze–thaw cycles. Samples were frozen and stored for 24 h at −80 °C, after which the samples were removed from the freezer to thaw them at room temperature under normal light. This cycle was repeated another two times after which SB202190 concentrations were determined as decribed before.

2.7. Pharmacokinetics of SB202190

The animal experiments were approved by the Animal Ethics Committee of the University of Groningen and licensed under number D4266A. In brief, male Wistar rats ($n = 3$; 280–300 g) were anesthesized with hypnorm/valium (Janssen-Cilag Ltd., Saunderton, UK) and equipped with a cannula in the carotid artery for blood sampling. The animals remained under anesthesia during the course of the pharmacokinetic study. An intravenous bolus dose of SB202190 (5 mg/kg) was injected through the penile vein. SB202190 was dissolved in 20% hydroxypropyl β-cyclodextrin solution (pH 3.0) at the concentration of 2.5 mg/ml using 5% DMSO. Blood samples (500 μl) were collected from the cannulated carotid artery at 2, 15, 30, 60, 120 and 240 min after injection. Kidneys and urine (from urinary bladder) were collected at 240 min after sacrificing the animals. Serum was obtained by allowing the blood samples to stand for 1 h on ice, after which the samples were centrifuged at 13,000 rpm for 5 min. Kidneys were homogenized in 0.1 M phosphate buffer saline (pH 7.2) in 1:3 (w/v) using ultra-turrax-T25 apparatus (20,000 rpm, 1 min). All the samples were stored at −80 °C until analysis of SB202190, as described above. Pharmacokinetic analysis of SB202190 concentrations in serum was performed using the Multifit program (Department of Pharmacokinetics and Drug Delivery, University of Groningen, The Netherlands). Goodness-of-fit was evaluated from visual inspection of the measured and calculated data points and of the residuals plotted against time and against concentration. The choice between one- and two-compartment models was based on the lowest value of the Akaike's Information Criterion (AIC).

3. Results and discussion

3.1. Method development

In the present study, we have developed a straightforward and accurate HPLC method to detect SB202190 in serum, kidney homogenates and urine. The extracted samples of SB202190 along with the I.S. were successfully separated on a C_8 analytical column. We tested several mobile phases at different pHs and found acetonitrile:water:trifluoroacetic acid (30:70:0.1, v/v/v; pH 2.0) as an appropriate mobile phase for the separation of the compounds within a run-time of 15 min. Both SB202190 and the I.S. were detected at 350 nm in the acidic conditions of the eluent, which allowed the straightforward detection of these compounds in the reconstituted extracts from the biological matrixes. Typical HPLC chromatograms of SB202190 after extraction from spiked serum, kidney homogenate and urine are shown in Fig. 2A–C, respectively. No interfering peaks of endogenous compounds were found at the retention times of SB202190 and the I.S.

3.2. Method validation

The average recoveries of SB202190 after the extraction procedure ranged from 74% from serum (0.25–5 μg/ml) and 75% from kidney homogenates (1–20 μg/g of tissue) to 92% from urine (1–20 μg/ml). In addition, the % recoveries of the I.S. in these matrixes were also found to be comparable with SB202190 (Table 1). A linear relationship was found between peak heights and drug concentrations within the ranges of 0.25–5 μg/ml for serum, 1–20 μg/g of tissue for kidney homogenate and 1–20 μg/ml for urine. The mean (±S.D.) regression equation for calibration curves in serum was $(0.000221 \pm 0.000011)C + (-0.0113 \pm 0.0242)$, $r^2 = 0.9931 \pm 0.0042$. The mean (±S.D.) regression equations for calibration curves in kidney homogenates and urine were $(0.0575 \pm 0.00703)C \pm (0.00067 \pm 0.0153)$, $r^2 = 0.9957 \pm 0.0028$ and

Table 1
Recoveries of SB202190 and the I.S. from serum, kidney and urine samples after spiking the samples with several concentrations of the drug

Compound	Serum		Kidney		Urine	
	μg/ml	Recovery (%), mean ± S.D. ($n = 6$)	μg/g	Recovery (%), mean ± S.D. ($n = 5$)	μg/ml	Recovery (%), mean ± S.D. ($n = 6$)
SB 202190	0.25	74.3 ± 15.6	1	76.67 ± 9.1	1	105 ± 8.6
	1	77.1 ± 5.1	4	71.54 ± 5.1	5	88.1 ± 1.6
	2	72.9 ± 14.9	8	80 ± 3.7	10	86.8 ± 2.9
	5	71.4 ± 14.2	20	70 ± 4.2	20	86.5 ± 1.8
I.S.	5	72.6 ± 9.5	20	83.3 ± 3.1 ($n = 6$)	20	101.7 ± 2.5

Table 2
Intra- and inter-day precision and accuracy of SB202190 measurements in rat serum

Concentrations (μg/ml)	Intra-day			Inter-day		
	Mean ± S.D. ($n = 6$)	Precision (% CV)	Accuracy (% bias)	Mean ± S.D. ($n = 6$)	Precision (% CV)	Accuracy (% bias)
0.25 (LLOQ)	0.25 ± 0.03	13.8	−0.30	0.27 ± 0.04	14.51	8.00
1	1.06 ± 0.09	8.35	6.08	1.00 ± 0.10	9.61	−0.29
2	1.90 ± 0.14	7.61	−5.08	2.09 ± 0.24	11.45	4.56
5	4.50 ± 0.47	10.43	−9.92	4.73 ± 0.27	5.64	−5.36

Table 3
Intra- and inter-day precision and accuracy of SB202190 measurements in kidney homogenates (per gram tissue)

Concentrations (μg/g)	Intra-day			Inter-day		
	Mean ± S.D. ($n = 5$)	Precision (% CV)	Accuracy (% bias)	Mean ± S.D. ($n = 6$)	Precision (% CV)	Accuracy (% bias)
1 (LLOQ)	1.09 ± 0.08	7.74	9.21	1.11 ± 0.14	12.45	11.39
4	4.08 ± 0.29	7.10	1.94	4.32 ± 0.29	6.64	7.95
8	8.22 ± 0.27	3.23	2.77	8.51 ± 0.45	5.25	6.40
20	19.89 ± 0.88	4.43	−0.53	19.85 ± 1.54	7.74	−0.75

$(0.0749 \pm 0.00363)C \pm (0.0144 \pm 0.0187)$, $r^2 = 0.9988 \pm 0.0019$, respectively. The coefficient of variations of slope for SB202190 in serum, kidney homogenates and urine were found to be <15% which indicates a high precision of the assay.

The intra- and inter-day variability of the assay for serum, kidney homogenates, urine are listed in Tables 2–4, respectively. Our data demonstrate that the method is reliable and reproducible since both % CV and % bias were below 15% for all estimated concentrations. Highest variation was observed in serum samples, most likely related to variability in the extraction efficiency. Since this occurred also at higher concentrations, we cannot contribute the variation to plasma protein binding of the drug. A possible improvement of the method may be achieved when serum samples are diluted first with physiological buffer, as was done with the urine samples to control pH. The LLOD and LLOQ of SB202190 were found to be 0.1 and 0.25 μg/ml in serum, 0.4 and 1 μg/g in kidneys and 0.5 and 1 μg/ml in urine, respectively.

3.3. Stability studies

The standard solutions of SB202190 and I.S. in methanol were found be stable since the % recoveries after 24 h at room temperature were 109 and 107%, respectively.

After a long-term stability study of 3 weeks, we found that SB202190 was stable in all the matrices since the % CV and % bias were below ±15% for higher concentrations and below ±20% for lower concentrations (Table 5). SB202190 was also found to be stable in serum and urine after three consecutive freeze–thaw cycles, but showed a significant decrease in kidney homogenate at 20 μg/g. Since no significant change was observed after a single thaw, we recommend that kidney

Table 4
Intra- and inter-day precision and accuracy of SB202190 in rat urine samples

Concentrations (μg/ml)	Intra-day			Inter-day		
	Mean ± S.D. ($n = 6$)	Precision (% CV)	Accuracy (% bias)	Mean ± S.D. ($n = 6$)	Precision (% CV)	Accuracy (% bias)
1 (LLOQ)	1.05 ± 0.08	7.34	5.40	0.92 ± 0.07	7.79	−8.13
5	4.77 ± 0.20	4.15	−4.54	4.99 ± 0.17	3.41	−0.13
10	9.20 ± 0.23	2.51	−7.99	9.90 ± 0.41	4.18	−1.01
20	18.69 ± 0.55	2.94	−6.56	19.39 ± 0.57	2.95	−3.06

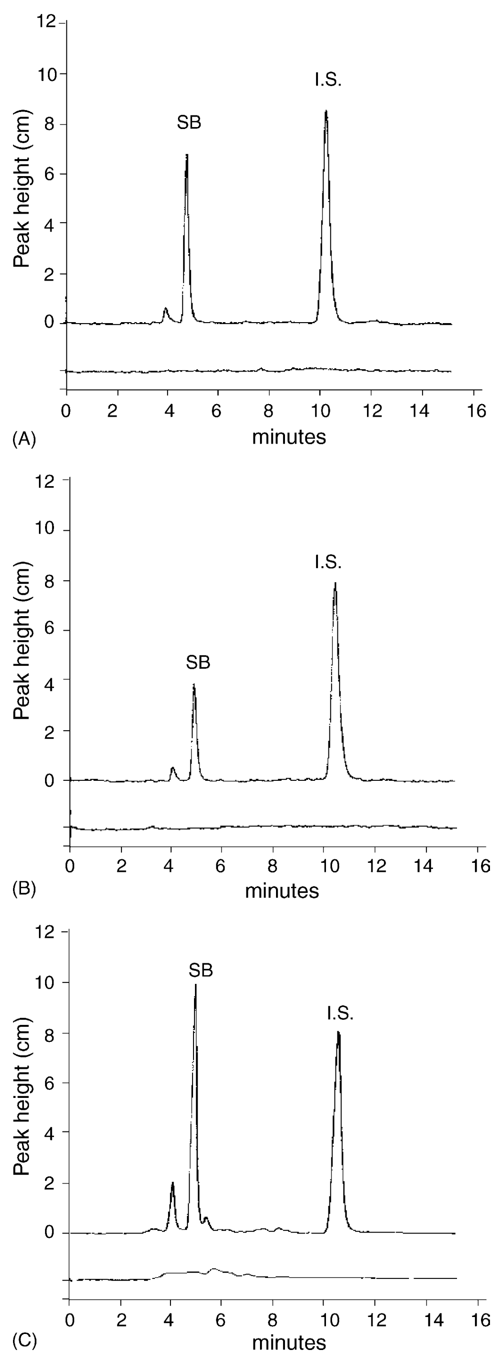

Fig. 2. Typical HPLC chromatograms of SB202190 after extraction from spiked rat serum (A), kidney homogenate (B) and urine (C). Upper lines in each figure represent spiked samples, while blank samples are shown at the bottom. Peaks of SB202190 and the internal standard are indicated by SB and I.S., respectively.

homogenate samples containing SB202190 should be stored in aliquots to avoid multiple freezing and thawing.

3.4. Pharmacokinetic study of SB202190 in rats

We applied the method for a pharmacokinetic study in which SB202190 was administered to healthy rats. A representative chromatogram of SB202190 in serum of one of the animals is shown in Fig. 3. After a single intravenous bolus injection of 5 mg/kg of SB202190, we monitored the drug concentrations in serum until 240 min after administration (Fig. 4). One-compartment and two-compartment models were tested for the best-fit according to AIC. The calculated pharmacokinetic parameters are listed in Table 6. Our experiments show

Table 5
Stability of SB202190 in serum, kidney and urine after 3 weeks at −80° and after multiple freeze–thaw cycles

	μg/ml or μg/g	Mean ± S.D.	% CV	% Bias
Long-term stability				
Serum ($n = 3$)	0.25	0.29 ± 0.01	4.49	16.2
	5	4.89 ± 0.3	6.13	−2.14
Kidney ($n = 3$)	1	1.14 ± 0.19	17.6	11.4
	20	18.6 ± 0.52	2.80	−7.12
Urine ($n = 4$)	1	1.0 ± 0.15	14.7	0.22
	20	20.8 ± 1.41	6.78	3.92
Three freeze–thaw cycles				
Serum ($n = 3$)	0.25	0.25 ± 0.02	9.62	−1.88
	5	4.89 ± 0.48	9.76	2.29
Kidney ($n = 4$)	1	1.11 ± 0.17	15.2	10.9
	20	16.9 ± 0.48	2.86	−15.3
Urine ($n = 3$)	1	0.96 ± 0.08	8.15	−4.22
	20	18.0 ± 0.64	3.56	−9.97

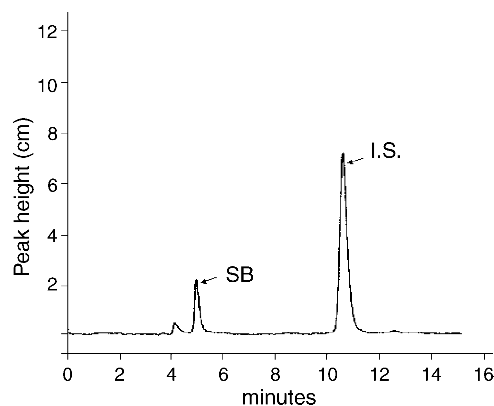

Fig. 3. HPLC chromatogram of SB202190 (SB) and the internal standard (I.S.) obtained from the extracts of the serum collected from a rat at 15 min after the intravenous injection of SB202190 (5 mg/kg). Internal standard (5 μg/ml) was added in the samples before extraction.

Table 6
Pharmacokinetic parameters (two-compartment model) derived from serum concentration–time curves after a single intravenous dose of 5 mg/kg of SB202190 in rats

Parameters	Values (mean ± S.E.M.)
V_{ss} (ml/kg)	4343 ± 400
CL (ml/kg/min)	72.2 ± 4.2
$AUC_{(0-\infty)}$ (min μg/ml)	69.2 ± 4.0
$t_{1/2}\ \alpha$ (min)	4.3 ± 1.7
$t_{1/2}\ \beta$ (min)	48.3 ± 6.8
MRT (min)	60.1 ± 6.7

V_{ss}: volume of distribution at steady state level; CL: plasma clearance; $AUC_{(0-\infty)}$: area under the curve from zero to infinity; $t_{1/2}\ \alpha$: distribution half life; $t_{1/2}\ \beta$: elimination half life; MRT: mean residence time.

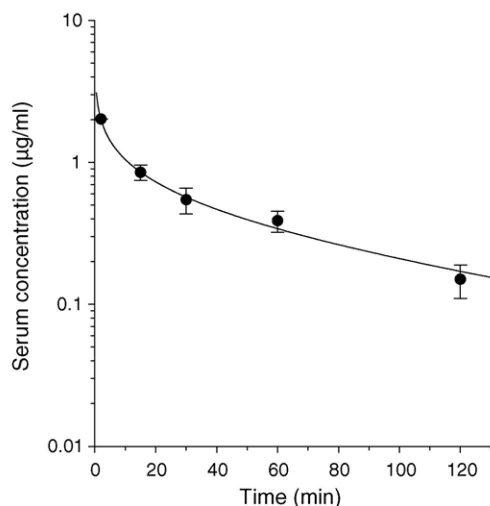

Fig. 4. Serum concentration–time disappearance curve of SB202190 in rats. Filled circles and the line depict the serum levels and the fitted pharmacokinetic data of SB202190, respectively. Data represent the mean ± standard deviation.

that SB202190 is distributed rapidly over a large initial volume of distribution of 2002 ± 292 ml/kg after which 50% of the compound is eliminated in approximately 50 min. These data are in good agreement with the pharmacokinetic parameters of SB203580, a p38 MAPK inhibitor which has a similar structure as SB202190 [10].

We also monitored SB202190 concentrations in kidneys and urine after sacrificing the animals, i.e. at 240 min after administration of the compound. Drug levels in kidney and urine samples were found to be below LLOQ, while one rat had minor amounts of SB202190 in the urine, corresponding to less than 0.3% of the injected dose. These results indicate that SB202190 is not eliminated via the renal route. Most likely, this type of compounds is eliminated via the liver, either by metabolism or excretion into the bile.

In future studies, we will investigate whether SB202190 can be delivered to the kidneys for the purpose of treating renal fibrosis. The present pharmacokinetic study with non-targeted SB202190 suggests that such a delivery strategy is required to achieve adequate renal concentrations of this drug. We have previously shown that the low molecular weight protein lysozyme can be applied as renal-specific drug carrier [11], and we will employ this drug targeting approach for SB202190 using SB202190-lysozyme conjugates.

4. Conclusion

The present HPLC method provides a new simple, reproducible and validated assay for the determination of SB202190 in serum, kidney homogenate and urine. This HPLC method will help in performing future experiments with renal delivered SB202190.

Acknowledgement

We are grateful to Mrs. A.M. van Loenen-Weemaes for her assistance in the animal experiments.

References

[1] H. Hanafusa, J. Ninomiya-Tsuji, N. Masuyama, M. Nishita, J. Fujisawa, H. Shibuya, K. Matsumoto, E. Nishida, J. Biol. Chem. 274 (1999) 27161.
[2] K. Ono, J. Han, Cell Signal. 12 (2000) 1.
[3] J.C. Lee, S. Kumar, D.E. Griswold, D.C. Underwood, B.J. Votta, J.L. Adams, Immunopharmacology 47 (2000) 185.
[4] C. Stambe, R.C. Atkins, G.H. Tesch, T. Masaki, G.F. Schreiner, D.J. Nikolic-Paterson, J. Am. Soc. Nephrol. 15 (2004) 370.
[5] S. Ramachandiran, Q. Huang, J. Dong, S.S. Lau, T.J. Monks, Chem. Res. Toxicol. 15 (2002) 1635.
[6] E. Tsiani, P. Lekas, I.G. Fantus, J. Dlugosz, C. Whiteside, Am. J. Physiol. Endocrinol. Metab. 282 (2002) E161.
[7] A. Masamune, M. Satoh, K. Kikuta, Y. Sakai, A. Satoh, T. Shimosegawa, J. Pharmacol. Exp. Ther. 304 (2003) 8.
[8] H. Ungefroren, W. Lenschow, W.B. Chen, F. Faendrich, H. Kalthoff, J. Biol. Chem. 278 (2003) 11041.
[9] Food and Drug Administration (FDA), Guidance for Industry in Bioanalytical Method Validation, Rockville, MD, USA, 2001.
[10] K.W. Ward, J.W. Prokscht, L.M. Azzaranot, J.A. Mumawa, T.J. Roethke, G.J. Stelman, M.J. Walsh, K.S. Zeigler, J.E. Surdy-Freed, J.R. Kehlert, J. Chokshi, M.A. Levy, B.R. Smith, Xenobiotica 31 (2001) 783.
[11] J. Prakash, A.M. van Loenen-Weemaes, M. Haas, J.H. Proost, D.K. Meijer, F. Moolenaar, K. Poelstra, R.J. Kok, Drug Metab. Dispos. 33 (2005) 683.