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High-performance liquid chromatographic analysis of a selective cyclooxygenase-1 inhibitor SC-560 in rat serum: application to pharmacokinetic studies

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Abstract

A method of analysis of SC-560 (5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-(trifluoromethyl)-1H-pyrazole) in biological fluids is necessary to study the kinetics of in vitro and in vivo metabolism. A simple high-performance liquid chromatographic method was developed for simultaneous determination of SC-560 and other products of metabolism in rat serum. Serum (0.1 ml) was precipitated with acetonitrile after addition of the internal standard, testosterone 17-propionate. Separation was achieved on a C_8 column with UV-detection at 240 nm. The calibration curve was linear ranging from 0.02 to 100 μg/ml. The mean recovery was >86.7%. Precision of the assay was <10% (R.S.D.%), and was within 15% at the limit of quantitation (20 ng/ml). Bias of the assay was lower than 15.5%. The limit of detection was 10 ng/ml for a 0.1 ml sample. The assay was applied successfully to the in vivo kinetic study of SC-560 in rats.

Keywords: Reversed-phase HPLC; UV-detection; Kinetics; Cyclooxygenase-1

1. Introduction

The discovery of two isoforms of cyclooxygenase (COX) has lead to the development of selective inhibitors of each isoform. SC-560 is a diaryl hetorocycle selective inhibitor of cyclooxygenase-1 (Fig. 1) [1]. Using human recombinant enzymes, the IC_{50} values for SC-560 are 9 nM and 6.4 μM for COX-1 and COX-2, respectively [1]. SC-560 is orally active in the rat, where 10 mg/kg completely abolishes the ionophore production of thromboxane B_2 in whole blood [2]. SC-560 has been used in many pharmacological and toxicological studies as a COX-1 inhibitor [3–6]. The use of this compound in pharmacological studies has been undertaken with a complete absence of knowledge of its pharmacokinetics. SC-560 is a structurally related to celecoxib, a selective inhibitor of COX-2. SC-560 like celecoxib has poor aqueous solubility and thus its pharmacokinetics and in particular its oral bioavailability may be an important determinant of its onset and duration of action [7]. Interestingly, some investigators administer this compound in 0.25% methylcellulose, hydoloxy propylcellulose or 1% methylcellulose without knowledge

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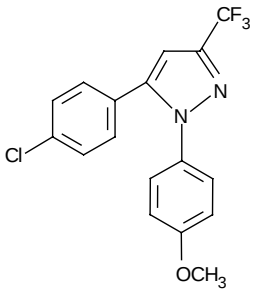

Fig. 1. Structure of SC-560.

of the impact on extent of oral bioavailability of these dosage forms or pharmacodynamic effects [4,5]. There is no information on whether the experimental disease states that SC-560 is administered in also affect the pharmacokinetics of this compound.

Currently, there are no validated analytical methods to quantify SC-560 and its metabolites in biological matrices. Hence, there is no pharmacokinetic data on the rate and extent of its metabolism in animal tissues that may determine the selectivity, efficacy and toxicity of SC-560.

In order to elucidate the metabolism kinetics of SC-560 and understand pharmacokinetic/pharmacodynamic relationships of this COX-1 inhibitor, knowledge of its metabolism pathways in biological fluids is of considerable importance. To our knowledge, no study has been published characterizing the pharmacokinetics of SC-560 described in the literature. Before performing pharmacokinetic/pharmacodynamic studies of SC-560 development of a selective and sensitive assay is necessary. No high-performance liquid chromatographic assays have been reported for the determination of SC-560.

The present study describes a selective, isocratic reversed-phase high-performance liquid chromatography (HPLC) method for the determination of SC-560 in rat serum and its application to in vivo kinetic studies.

2. Experimental

2.1. Chemicals and reagents

SC-560 was purchased from Cayman Chemicals (Ann Harbour Mo., USA). HPLC grade methanol, acetonitrile, and water were purchased from J. T. Baker (Phillipsburg, NJ, USA). Testosterone 17-propionate was obtained from Sigma Chemicals (St. Louis, MO, USA). Polyethylene glycol 600 was purchased from Union Carbide Chemicals (Danbury, CT, USA). Rats were obtained from Charles River Laboratories.

2.2. Chromatographic system and conditions

The HPLC system used was a Shimadzu HPLC (Kyoto, Japan), consisting of an LC-10AT *VP* pump, an SIL-10AF auto-injector, an SPD-M10A *VP* spectrophotometric diodearray detector, and a SCL-10A *VP* system controller. Data collection and integration were accomplished using Shimadzu EZStart 7.1.1 SP1 (Kyoto, Japan).

The analytical column used was Beckman ultrasphere octyl column (150 mm × 2 mm ID, 5 μm particle size, Beckman Instrument, Fullerton, CA, USA) equipped with a pre-column (7.5 mm × 2 mm ID, 5 μm) of the same packing material. The mobile phase consisted of methanol and water (7:3, v/v), filtered and degassed under reduced pressure, prior to use. Separation was carried out isocratically at ambient temperature (25 ± 1 °C), and a flow rate of 0.25 ml/min, with UV-detection at 240 nm.

2.3. Stock and working standard solutions

A target amount of 20 mg of SC-560 was accurately weighed on an analytical balance (AG245, Mettler Instrument Co, Hightstown, NJ, USA) and dissolved with methanol in a 10 ml volumetric flask to make a stock standard solution in methanol with a target concentration of 2 mg/ml. A methanolic stock solution of testosterone 17-propionate (internal standard) was prepared similarly with the target concentration of 1 mg/ml. This solution was diluted with methanol to make a working internal standard solution of 10 μg/ml. These solutions were protected from light and stored at −20 °C between use, for no longer than 3 months. Calibration standards in serum were prepared from the stock solution of SC-560 by sequential dilution with blank rat serum, yielding a series of concentrations namely 0.02, 0.05, 1.0, 3.0, 10.0, 30.0 and 100.0 μg/ml, in three replicates.

Quality control (QC) samples were prepared from the stock solution of SC-560 by dilution with blank rat

serum to yield target concentrations of 0.02, 0.05, 0.3, 1.0, 3.0, 10.0 and 100.0 μg/ml. The QC samples were prepared independently from the calibration standards and divided into 0.1 ml aliquots in micro centrifuge tubes and stored at −70 °C before use.

2.4. Sample preparation

To working standards or samples (0.1 ml) was added 50 μl of internal standard solution (10 μg/ml) and 1 mL ice-cold acetonitrile. The mixture was vortexed for 1 min (Vortex Genie-2, VWR Scientific, West Chester, PA, USA), and centrifuged at 15000 rpm at 4 °C for 5 min (Beckman Microfuge, Beckman Coulter, Inc., Fullerton, CA, USA). The supernatant was collected and evaporated to dryness using a Heto Vac concentrator (Heto-Holten, DK-3450 Allerød, Danmark). The residue was reconstituted with 100 μl of 70% methanol (v/v), vortexed for 1 min and centrifuged at 8000 rpm at 4 °C for 5 min, and 40 μl of the supernatant was injected onto the column.

2.5. Precision and accuracy

The within-run precision and accuracy of the replicate assays ($n = 6$) were tested by using seven different concentrations, namely 0.02, 0.05, 0.3, 1.0, 3.0, 10.0 and 100.0 μg/ml. The between-run precision and accuracy of the assays were estimated from the results of six replicate assays of QC samples on 6 different days within 1 week. The precision was evaluated by relative standard deviation (R.S.D.%). The accuracy was estimated based on the mean percentage error of measured concentration to the actual concentration [8].

2.6. Recovery

Recovery for SC-560 from rat serum was assessed ($n = 6$) at 0.02, 0.05, 0.1, 1, 10 and 100 μg/ml. A known amount of SC-560 was spiked into 0.1 ml rat serum to give the above concentrations. The samples were treated as described under Section 2.4 and analyzed by HPLC. The recovery was determined by comparing the peak areas of SC-560 to those of SC-560 solutions of corresponding concentration injected directly in the HPLC system without extraction.

2.7. Freeze–thaw stability of SC-560 samples

The freeze–thaw stability of SC-560 was evaluated at seven concentrations 0.02, 0.05, 0.3, 1, 3, 10 and 100 μg/ml, using QC samples. These samples were analyzed in triplicate without being frozen at first, and then stored at −70 °C and thawed at room temperature (25 ± 1 °C) for three cycles.

The stability of SC-560 in reconstituted extracts during run-time in the HPLC auto-injector was investigated, using pooled extracts from QC samples of six concentration levels 0.05, 0.3, 1, 3, 10 and 100 μg/ml. Samples were kept in the sample rack of the auto-injector and injected into HPLC system every 3 h, from 0 to 12 h at the temperature of auto-injector (26 ± 1 °C).

2.8. Pharmacokinetics of SC-560 in rat

Male Sprague Dawley rats (150–200 g) were anaesthetized using halothane and a silastic catheter was cannulated into the right jugular vein. Animals were placed in metabolic cages, allowed to recover overnight and fasted for 12 h before dosing. On the day of experiments, animals were dosed orally with SC-560 (10 mg/kg) in polyethylene glycol 600 using gastric gavage. Serial blood samples (0.25 ml) were collected at 0, 0.5, 1, 2, 4, 6, 8, and 12 h. After each sample collection, the cannula was flushed with 0.25 ml of saline. Following centrifugation of the blood samples, serum was collected and stored at –70°C until analyzed.

2.9. Data analysis

Quantification was based on calibration curves constructed using peak area ratio (PAR) of SC-560 to internal standard, against SC-560 concentrations using unweighted least squares linear regression. Pharmacokinetic parameters were estimated using WinNonlin (version 1.0).

3. Results and discussion

3.1. Chromatography

SC-560 and the internal standard were eluted at 14.9 and 18.8 min without interfering peaks

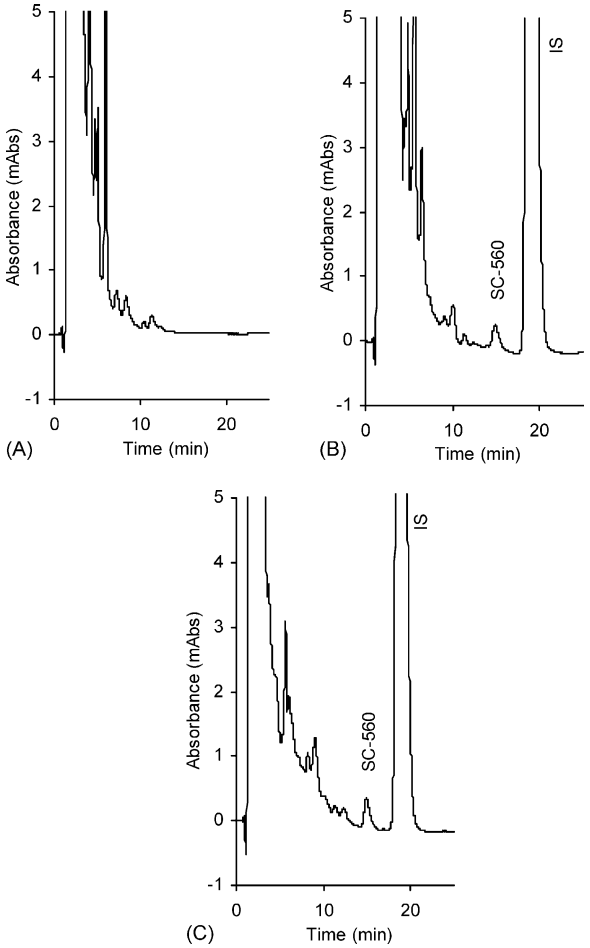

Fig. 2. Representative chromatograms, of (A) drug-free rat serum, (B) serum containing SC-560 (0.05 μg/ml) and the internal standard (IS, 10 μg/ml), and (C) serum sample from a rat following oral administration of SC-560 (10 mg/kg) for 6 h.

co-eluted under current HPLC conditions (Fig. 2A and B).

The performance of the HPLC assay was assessed using the following parameters, namely peak shape, interference from endogenous substances in rat serum, linearity, limit of quantitation (LOQ), limit of detection (LOD), freeze–thaw stability, stability of reconstituted extracts, precision, accuracy and recovery. Various conditions of HPLC were tested to achieve the best resolution between SC-560 and other interfering peaks. The volume of the mobile phase for reconstitution was found to be critical to achieve a relatively cleaner background. The optimal volume of reconstitution was 100 μl.

Based on the wavelength maximum of SC-560 in the mobile phase, the UV detector in this study was set at 240 nm.

3.2. Linearity, LOQ and LOD

An excellent linear relationship ($r^2 = 0.9999$) was demonstrated between PAR of SC-560 to internal standard and the corresponding serum concentrations of SC-560 over a range of 0.02–100 μg/ml. The mean regression line from the validation runs was described by SC-560 (μg/ml) = PAR × 5.3644 + 0.0057. The LOQ of this assay was 0.02 μg/ml in rat serum with the corresponding relative standard deviation and bias of 12.3 and −13.1%, respectively, which was determined by analyzing six replicates of spiked sample at this concentration. The back-calculated concentration of QC samples was within the acceptance criteria. The LOD of SC-560 was estimated to be 0.01 μg/ml in rat with the signal to noise ratio ≥ 3:1.

3.3. Precision, accuracy and recovery

The within- and between-run R.S.D.% calculated during replicate assays ($n = 6$) of SC-560 in rat serum was <15% over a wide range of SC-560 concentrations (Table 1). The intra- and inter-run bias assessed during replicate assays varied between −15.5 and 4.5% (Table 1). Precision and accuracy studies indicated that the developed HPLC method is reproducible and accurate. The mean recovery of SC-560 from rat serum varied from 86.7 to 94.3% (Table 2). High recovery of SC-560 from rat serum suggested that there was negligible loss during the protein precipitation process.

3.4. Stability of SC-560 samples

No significant degradation was detected after the samples of SC-560 in rat serum following three freeze–thaw circles. The recoveries were within 91.4 and 105.3% following three freeze–thaw cycles for SC-560 QC samples of 0.02, 0.05, 0.3, 1, 3, 10 and 100 μg/ml. There was no significant decomposition observed after the reconstituted extracts of SC-560 were stored in the auto-injector at room temperature

Table 1
Within- and between-run precision and accuracy for SC-560 assay in rat serum

Concentration (μg/ml), actual	Within-run ($n = 6$)			Between-run ($n = 6$)		
	Observed	R.S.D.%	Bias%	Observed	R.S.D.%	Bias%
0.02	0.017	13.2	−15.5	0.017	4.7	−14.4
0.05	0.047	7.5	−5.8	0.048	4.1	−3.5
0.3	0.297	3.2	−1.0	0.301	4.0	0.3
1	1.02	4.3	1.8	1.04	6.3	4.5
3	2.78	4.0	−7.3	2.92	5.8	−2.7
10	9.67	1.7	−3.3	10.00	3.9	0.0
100	102.0	2.3	2.0	100.1	2.6	0.1

for 12 h. The measurements were from 96.4 to 101.2, 97.5–98.6, 98.7–99.2, 99.8–100.3, and 99.4–99.8% of the initial value for extracts of SC-560 samples of 0.3, 1, 3, 10 and 100 μg/ml respectively, during the storage in the auto-injector at room temperature for 12 h.

3.5. Pharmacokinetics of SC-560 in rat

The HPLC method has been applied to the determination of SC-560 in the pharmacokinetic study of SC-560 in rat. Following oral administration of SC-560, a slow absorption of SC-560 was observed with t_{max} of 4 h. A terminal elimination half-life of about 5 h was evident (Fig. 3).

In summary, the developed HPLC assay is selective, reproducible and accurate. It has been successfully applied to the study of pharmacokinetics of SC-560 in rats.

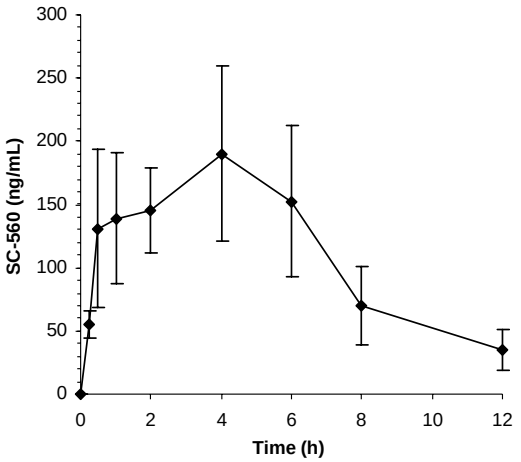

Fig. 3. Concentration time profile of SC-560 following the administration of SC-560 orally (10 mg/kg) to rats (mean ± S.E., $n = 5$).

Table 2
Recovery of SC-560 from rat serum at various concentrations ($n = 6$)

Concentration (μg/ml)	Mean recovery %	R.S.D.%
0.02	88.6	8.9
0.05	90.0	5.6
1	86.7	8.7
10	94.3	4.8
100	90.9	3.9

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References

[1] C.J. Smith, Y. Zhang, C.M. Koboldt, J. Muhammad, B.S. Zweifel, A. Shaffer, J.J. Talley, J.L. Masferrer, K. Seibert, P.C. Isakson, Proc. Natl. Acad. Sci. USA 95 (1998) 13313–13318.
[2] J.L. Masferrer, B.S. Zweifel, P.T. Manning, Proc. Natl. Acad. Sci. 91 (1994) 3228–3232.
[3] J.L. Wallace, W. McKnight, B.K. Reuter, N. Vergnolle, Gastroenterology 119 (2000) 706–714.
[4] R.J. Sawdy, D.M. Slater, W.J. Dennes, M.H. Sullivan, P.R. Bennett, Placenta 21 (2000) 54–57.
[5] B. Gretzer, N. Maricic, M. Respondek, R. Schuligoi, B.M. Peskar, Brit. J. Pharmacol. 132 (2001) 1565–1573.
[6] A. Tanaka, S. Hase, T. Miyazawa, K. Takeuchi, J. Pharmacol. Exp. Ther. 300 (2002) 754–761.
[7] N.M. Davies, A.J. McLachlan, R.O. Day, K.M. Williams, Clin. Pharmacokinet. 38 (2000) 225–242.
[8] V.P. Shah, K.K. Midha, S. Dighe, I.J. McGilveray, J.P. Skelly, A. Yacobi, T. Layloff, C.T. Viswanathan, C.E. Cook, R.D. McDowall, K.A. Pittman, S. Spector, Int. J. Pharm. 82 (1992) 1–7.