Journal of Pharmaceutical and Biomedical Analysis 55 (2011) 1104–1110

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Simultaneous determination of a p38 MAP kinase inhibitor and its amide hydrolyzed metabolite in Cynomolgus monkey plasma by LC–MS/MS, and its application to a toxicokinetic study

Anna Akrami, Faye Hsieh, Valerie Almon, Bernd A. Bruenner, Christopher James, Philip Wong*

Department of Pharmacokinetics and Drug Metabolism, Amgen, Thousand Oaks, CA 91320-1799, United States

ARTICLE INFO

Article history:
Received 3 February 2011
Received in revised form 10 March 2011
Accepted 12 March 2011
Available online 21 March 2011

Keywords:
Inflammation
p38 MAP kinase inhibitor
LC–MS/MS

ABSTRACT

A LC–MS/MS method was developed for the determination of a p38 MAP kinase inhibitor (Compound I) and its amide hydrolyzed metabolite (M7) in Cynomolgus monkey plasma over the concentration range of 1.00–1000 ng/mL. Stable isotope labeled compounds (d_3-Compound I and d_3-M7) were used as internal standards (IS). Samples were prepared using protein precipitation in the 96-well format with a 30 μL plasma sample volume. Chromatographic separation was performed with reversed-phase liquid chromatography on a Varian Monochrom C_{18} (100 mm × 2.00 mm, 5 μm) analytical column. The mobile phases were 5 mM ammonium formate in acetonitrile/water (95/5, v/v) pH 7.0 and 5 mM ammonium formate in acetonitrile/water (5/95, v/v) pH 7.0. Gradient elution, at a flow rate of 550 μL/min, was used to separate Compound I and M7. Positive atmospheric pressure chemical ionization was utilized with detection by multiple reaction monitoring (MRM). Total run time was about 3.2 min. This method was validated following the current Food and Drug Administration (FDA) guidance for bioanalytical method validation. The intra- and inter-day precision (% CV) and accuracy (% bias) at all concentrations tested were below 15% for both analytes. The mean recoveries for Compound I, M7, d_3-Compound I, and d_3-M7 were 106%, 107%, 108% and 105%, respectively. The method was successfully applied to support a GLP toxicokinetic study in Cynomolgus monkeys after oral administration of Compound I. A total of 48 samples (~12.5% of the total number of samples) were selected for incurred sample reanalysis (ISR). The % difference between the reassay concentrations and the original concentrations were all less than 20% of their mean values and met the acceptance criteria for ISR.

1. Introduction

Mitogen-activated protein (MAP) kinases are important components of intracellular signaling cascades that become activated in response to certain extracellular stimuli such as ultraviolet radiation and osmotic shock [1,2]. The p38 kinase is a member of the MAP kinase family [3,4] and is considered as a key enzyme mediating the pro-inflammatory response [3–5]. In recent years, p38 MAP kinase has been studied extensively as a possible therapeutic target for the treatment of various inflammatory diseases, such as arthritis and other joint diseases [6–9]. Several p38 MAP kinase inhibitors have been developed as anti-inflammatory therapeutics [8]. In addition, these inhibitors have been proposed for the treatment of end-stage organ diseases such as renal fibrosis [10].

Compound I is a potent p38 MAP kinase inhibitor and the structure is shown in Fig. 1A. In vitro rat, monkey and human liver hepatocyte incubation studies indicated that M7 was potentially the major metabolite (>30% of parent) of Compound I (unpublished data); the structure of M7 is shown in Fig. 1B. Recent guidance regarding metabolite in safety testing (MIST) emphasizes the importance of monitoring major metabolites in GLP preclinical and clinical studies [11]. This report therefore describes a validated LC–MS/MS method for the simultaneous determination of Compound I and M7 in Cynomoglus monkey plasma. The method used protein precipitation to prepare samples in a 96-well format and stable isotope labeled compounds (d_3-Compound I and d_3-M7) were used as the internal standards (IS) for both analytes (structures are shown in Fig. 1C and D). The method was applied to a GLP toxicokinetic study in Cynomolgus monkeys after oral administration of Compound I.

* Corresponding author at: Amgen, Pharmacokinetic and Drug Metabolism, One Amgen Center Drive, Thousand Oaks, CA 91320-1799, United States.
Tel.: +805 313 4701; fax: +805 499 4868.
E-mail address: pwong@amgen.com (P. Wong).

0731-7085/$ – see front matter
doi:10.1016/j.jpba.2011.03.022

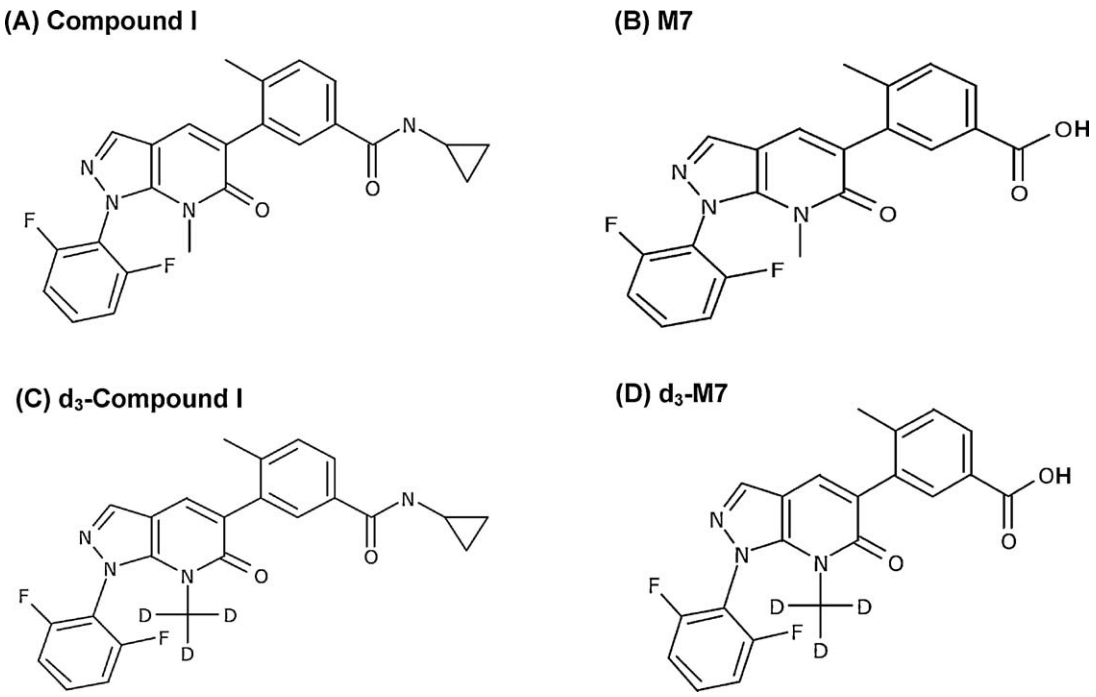

Fig. 1. Structures of (A) Compound I, (B) M7, (C) d_3-Compound I and (D) d_3-M7.

2. Experimental

2.1. Chemicals and reagents

Compound I, M7, and the deuterated internal standards were synthesized at Amgen (Thousand Oaks, CA). HPLC grade methanol, acetonitrile (ACN) and water were purchased from Burdick and Jackson. Dimethyl sulfoxide (DMSO), ammonium formate and formic acid were purchased from Sigma–Aldrich. Control K_2EDTA Cynomolgus monkey plasma and plasma with approximately mid- and high levels of hemolysis (Becton Dickinson Relative Hemolysis Scale of ~140 and 1110 mg/dl, respectively) [12] were supplied by Bioreclamation Inc. (East Meadow, NY).

2.2. Equipment and LC–MS/MS conditions

The HPLC system consisted of a Shimadzu LC-20ADXR Prominence pump and a SIL-20ACXR Prominence autosampler with

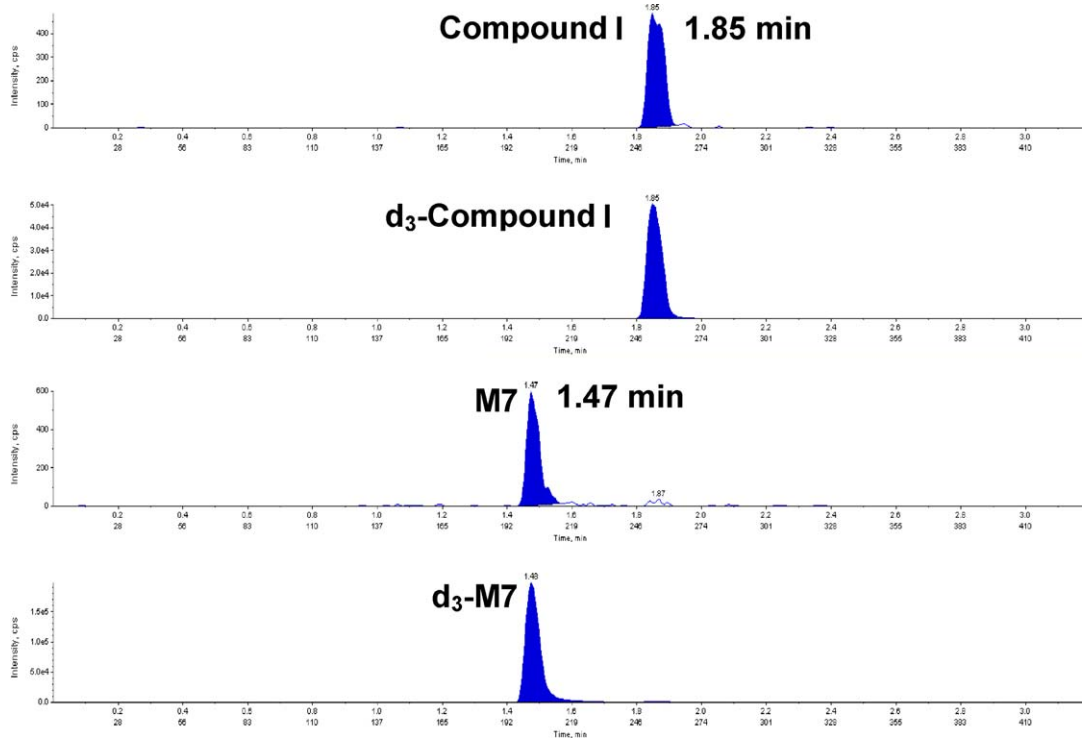

Fig. 2. Representative chromatogram at the lower limit of quantitation (LLOQ, 1.00 ng/mL).

Table 1
Calibration curve parameters for the determination of Compound I and M7 in Cynomolgus monkey plasma.

Batch number	Slope	Intercept	R-squared
Compound I			
1	0.010217	0.001087	0.9987
2	0.010101	0.000160	0.9989
3	0.010094	0.000963	0.9996
Mean	0.10137	0.000737	0.9991
% CV	0.7	68.2	0.1
M7			
1	0.002469	0.000151	0.9983
2	0.002491	0.000070	0.9988
3	0.002458	−0.000034	0.9991
Mean	0.002473	0.000062	0.9987
% CV	0.7	150.0	0.0

cooler and rack changer (Shimadzu Corporation, Kyoto, Japan). Samples were separated using reversed-phase liquid chromatography on a Varian Monochrom C_{18} column, 5 μm, 100 mm × 2.00 mm (Lake Forest, CA). The mobile phase consisted of 5 mM ammonium formate in acetonitrile/water (5:95) pH 7.0 (v/v) (mobile phase A) and 5 mM ammonium formate in acetonitrile/water (95:5) pH 7.0 (v/v) (mobile phase B). Flow rate was 550 μL/min. The gradient started at 15%B and increased to 95%B from 0.5 to 1.6 min, and was held constant until 2.6 min. At 2.6 min, the gradient was changed back to the starting conditions. The total run time was about 3.2 min. Mass spectrometric analysis was carried out using an API 4000 triple quadrupole mass spectrometer (AB Sciex, Foster City, CA) with an atmospheric pressure chemical ionization (APCI) interface operated in the positive ion mode. Detection was performed using multiple reaction monitoring (MRM) with transitions of m/z 435 → 334 for Compound I, m/z 396 → 334 for M7, m/z 438 → 334 for d_3-Compound I and m/z 399 → 334 for d_3-M7.

The dwell time for each MRM transition was 100 ms. The APCI source temperature and current were set at 400 °C and 3 μA, respectively. The curtain gas and collision gas were set at 20 and 4, respectively (arbitrary units).

Peak areas were integrated by the Sciex Analyst software, Version 1.4.1, residing on a Window NT computer. Following peak area integration, the data were imported into a Watson Laboratory Information Management System (version 7.0, Thermo Scientific, Philadelphia, PA) where concentrations were calculated using weighted ($1/x^2$) linear regression.

2.3. Standards, internal standards and quality control samples

Stock solutions (1.00 mg/mL) in DMSO were prepared from separate weightings of Compound I and M7 reference materials. A mixture of Compound I and M7 solution was used to prepare a working solution at 100 μg/mL in 1:1 ACN: H_2O. Serial dilutions of the mixed working solution yielded the spiking solutions for the preparation of calibration standards or QC samples. Separate weightings and solutions were always used to prepare calibration and QC samples. Spiking solutions in 1:1 ACN: H_2O were always prepared on the day of use. All solutions were stored at room temperature. Stock solutions of the internal standards d_3-Compound I and d_3-M7 at 1.00 mg/mL were prepared in DMSO. The stock IS solutions were then diluted with acetonitrile to prepare a mixture of 20 ng/mL of d_3-Compound I and 95 ng/mL of d_3-M7. All solutions were stored at room temperature.

QC samples at concentrations of 1.00 (LLOQ), 3.00 (low QC), 50.0 (medium QC), 800 (high QC) and 4000 ng/mL (dilution QC, 10×) were prepared by spiking Compound I and M7 into control K_2EDTA Cynomolgus monkey plasma. For example, 25 μL of 1000 ng/mL solution was spiked into the blank plasma (25 mL) to yield 1.00 ng/mL of the LLOQ QC samples. The QC samples were then aliquoted into polypropylene vials and stored in a freezer at −70 ± 10 °C until analysis.

Table 2
Intraday and interday accuracy and precision of Compound I and M7 QC samples.

Day	Statistic	Compound I QC sample concentration (ng/mL)				
		LLOQ 1.00	Low QC 3.00	Mid QC 50.0	High QC 800	Dilution QC (10x) 4000
1	Intraday mean (n = 6)	0.960	3.03	50.0	815	4070
	% CV	6.6	13.2	2.0	2.2	2.3
	% Bias	−4.0	1.0	0.0	1.9	1.8
2	Intraday mean (n = 6)	1.02	2.85	49.6	812	
	% CV	4.4	3.5	3.4	1.6	
	% Bias	2.0	−5.0	−0.8	1.5	
3	Intraday mean (n = 6)	0.923	3.01	49.8	809	
	% CV	6.5	1.8	1.5	2.8	
	% Bias	−7.7	0.3	−0.4	1.1	
1–3	Interday mean (n = 18)	0.969	2.96	49.8	812	
	% CV	7.1	8.1	2.3	2.1	
	% Bias	−3.1	−1.3	−0.4	1.5	

Day	Statistic	M7 QC Sample Concentration (ng/mL)				
		LLOQ1.00	Low QC3.00	Mid QC50.0	High QC800	Dilution QC (10x)4000
1	Intraday mean (n = 6)	0.975	3.03	49.1	806	4090
	% CV	6.6	5.6	2.7	1.5	1.5
	% Bias	−2.5	1.0	−1.8	0.8	2.3
2	Intraday mean (n = 6)	1.00	2.84	48.5	804	
	% CV	9.2	6.1	1.3	2.0	
	% Bias	0.0	−5.3	−3.0	0.50	
3	Intraday mean (n = 6)	1.02	2.96	49.9	804	
	% CV	6.0	4.5	1.4	2.3	
	% Bias	2.0	−1.3	−0.2	0.50	
1–3	Interday mean (n = 18)	0.997	2.94	49.2	804	
	% CV	7.2	5.8	2.2	1.8	
	% Bias	−0.30	−2.0	−1.6	0.5	

Table 3
Bench top, freeze-thaw and long-term stability of Compound I and M7 in Cynomolgus monkey plasma.

Stability conditions	Nominal concentration (ng/mL)	Mean concentration[a] (ng/mL)	% CV	% Bias
Compound I				
4-h bench top stability	3.00	3.04	4.6	1.3
	800	813	0.6	1.6
3 freeze-thaw cycles	3.00	2.96	2.4	−1.3
	800	805	4.1	0.6
183 days at −70 °C	3.00	2.79	7.4	−7.0
	800	747	0.3	−6.6
M7				
4-h bench top stability	3.00	3.01	3.4	0.3
	800	804	2.8	0.5
3 freeze-thaw cycles	3.00	2.84	4.7	-5.3
	800	805	2.6	0.6
183 days at −70 °C	3.00	2.81	5.7	−6.3
	800	780	3.4	−2.5

[a] All measurements were in triplicates except the freeze-thaw stability experiments where 6 replicates were used.

2.4. Sample preparation

For the preparation of calibration standards, 30 μL of spiking standard solutions were mixed with 30 μL of blank plasma in 1.2 mL cluster tubes. For QC, blank and study samples, 30 μL of plasma were mixed with 30 μL of 1:1 ACN: H_2O in 1.2 mL cluster tubes. All the samples were arranged in a 96-well block and 125 μL of the working internal standard solution was added, except for the double blanks to which 125 μL of ACN was added. Samples were then vortexed at medium speed for approximately 30 s and centrifuged at 3000 rpm (~1700 g) for 5 min. Using a Tomtec Quadra 3, approximately 140 μL of supernatant was transferred into a 96-well plate. Samples were dried down under nitrogen at 30 °C and then reconstituted with 125 μL of 95:5 mixture of mobile phase A: mobile phase B. The 96-well plate was then loaded into the Shimadzu autosampler with the sample compartment set to maintain a temperature of 13 °C, and 25 μL of the samples were injected into the LC–MS/MS system for analysis.

2.5. Method validation

2.5.1. Lower limit of quantitation (LLOQ) and calibration curves

LLOQ was defined as the lowest concentration on the calibration curve with an acceptable accuracy (% bias within ±20%) and precision (%CV ≤20%). Eight calibration standards were prepared in Cynomolgus monkey plasma at concentrations of 1, 2, 10, 20, 50, 100, 500 and 1000 ng/mL and run in duplicate at the beginning and end of each batch.

2.5.2. Accuracy and precision

Accuracy and precision were assessed by determining the concentrations of QC samples at four concentration levels (LLOQ, 1.00 ng/mL; low, 3.00 ng/mL; medium, 50.0 ng/mL;

Table 4
Mean (±SD, $n = 4$) toxicokinetic parameters of Compound I and M7 in Cynomolgus monkey plasma.

TK parametersCompound I	Day 1		Day 91	
	Male	Female	Male	Female
Group 2–(0.1 mg/kg per day)				
$AUC_{(0-24\,h)}$ (ng × h/mL)	400 (±21.1)	399 (±59.8)	498 (±64.1)	524 (±57.5)
C_{max} (ng/mL)	30.8(±2.48)	34.1 (±6.70)	45.5 (±5.71)	39.1 (±6.83)
T_{max} (h)	4.25 (±2.06)	3.00 (1.15)	1.75 (±0.50)	2.75 (±1.50)
Group 3–(1 mg/kg per day)				
$AUC_{(0-24\,h)}$ (ng × h/mL)	4430 (±948)	3350 (±814)	4510 (±604)	3340 (±657)
C_{max} (ng/mL)	356 (±53.8)	263 (±39.5)	338 (±46.4)	294 (±56.0)
T_{max} (h)	3.75 (±2.36)	4.00 (±0.00)	2.50 (±1.00)	2.25 (±1.26)
Group 4–(30 mg/kg per day)				
$AUC_{(0-24\,h)}$ (ng × h/mL)	27000 (±7470)	28500 (±4300)	25400 (±4940)	31300 (±4380)
C_{max} (ng/mL)	1670 (±442)	1720 (±196)	1590 (±200)	1870 (±349)
T_{max} (h)	5.50 (±1.64)	5.67 (±2.16)	2.40 (±0.89)	6.00 (±2.24)
M7	Day 1		Day 91	
	Male	Female	Male	Female
Group 2–(0.1 mg/kg per day)				
$AUC_{(0-24\,h)}$ (ng × h/mL)	120 (±38.0)	108 (±28.6)	266 (±77.7)	226 (±73.2)
C_{max} (ng/mL)	8.36 (±4.46)	7.88 (±1.68)	21.3 (±5.38)	16.6 (±6.79)
T_{max} (h)	4.00 (±0.00)	3.00 (±1.15)	3.00 (±2.71)	2.25 (±1.26)
Group 3–(1 mg/kg per day)				
$AUC_{(0-24\,h)}$ (ng × h/mL)	1210 (±277)	965 (±167)	2670 (±1110)	1870 (±322)
C_{max} (ng/mL)	77.7 (±14.3)	61.2 (±13.0)	192 (±105)	153 (±32.4)
T_{max} (h)	6.25 (±1.50)	5.75 (±2.50)	5.00 (±2.45)	1.75 (±0.50)
Group 4–(30 mg/kg per day)				
$AUC_{(0-24\ h)}$ (ng × h/mL)	10300 (±2840)	8540 (±2040)	17100 (±3970)	17400 (±7610)
C_{max} (ng/mL)	578 (±122)	475 (±115)	1180 (±331)	920 (±345)
T_{max} (h)	7.00 (±0.00)	9.83 (±6.94)	5.00 (±2.74)	5.80 (±2.68)

high, 800 ng/mL) using six replicates in three analytical batches.

2.5.3. Carryover

Carryover was evaluated by determining the peak areas of two control samples run after the injection of the upper limit of quantitation (ULOQ) calibration standard.

2.5.4. Matrix effect and selectivity

Matrix effect was evaluated with samples prepared from six different lots of plasma at the low QC concentration ($n = 3$ for each lot). Assay selectivity was also evaluated from samples prepared from six different lots of blank plasma, and each was analyzed with and without the internal standards.

2.5.5. Stability

Stability of the Compound I and M7 in plasma was determined under different storage and handling conditions. Bench top stability, freeze-thaw stability, autosampler reinjection reproducibility, and long term stability were evaluated at two QC levels (low and high QC concentrations). Bench top stability was assessed by analyzing samples kept at ambient temperature for 4 h prior to sample preparation. For freeze-thaw stability, QC samples were subjected to three freeze-thaw cycles consisting of thawing samples at room temperature for at least one hour, vortexing, and then refreezing for at least 12 h at $-70 \pm 10\,^{\circ}C$. After three freeze-thaw cycles, the QC samples were analyzed using freshly prepared calibration standards. Reinjection reproducibility was assessed by re-analyzing processed samples kept in the autosampler at 13 °C for 71 h. Long term stability was evaluated at $-70 \pm 10\,^{\circ}C$ for 183 days.

2.5.6. Extraction recovery

The recoveries of Compound I, M7, d_3-Compound I and d_3-M7 were evaluated at low, medium and high QC levels ($n = 6$). Recoveries were determined by comparing the ratio of the average peak areas of extracted QC samples (i.e. after protein precipitation with working IS) to the average peak areas of extracted blank plasma spiked with equivalent concentrations of Compound I, M7, d_3-Compound I and d_3-M7.

2.5.7. Hemolysis

Plasma with mid- and high levels of hemolysis (Section 2.1) were spiked at low and high QC concentrations and kept at $-70 \pm 10\,^{\circ}C$ for at least 12 h before analysis. The hemolysed QC samples were extracted and analyzed in triplicate.

2.5.8. Application to a toxicokinetic study

The *in vivo* toxicokinetic experiments were performed at Charles River Laboratories (Reno, Nevada), an American Association for Accreditation of Laboratory Animal Care (AAALA) accredited facility. The study protocol was reviewed by Charles River Laboratories Institutional Animal Care and Use Committee (IACUC) internal committee and conducted in accordance with all local guidelines. Compound I was dosed orally to 4 male and 4 female Cynomolgus monkeys (8 animals per group) in four groups (0, 0.1, 1 and 30 mg/kg per day) for 91 days. Blood samples were collected at 0, 1, 2, 4, 7 and 24 h post-dose on day-1 and day-91. All blood samples were processed within 30 min to obtain the plasma samples which were then shipped in dry ice to Amgen (Thousand Oaks, CA). The plasma samples were then stored at $-70 \pm 10\,^{\circ}C$ until analysis. Pharmacokinetic analysis of Compound I and M7 was performed by non-compartmental analysis using WinNonlin (v.5.1.1 Pharsight Corporation, Mountain View, CA).

2.5.9. Incured sample reanalysis

Reproducibility of the analytical method was further evaluated by re-analysis of incurred samples. A total of 48 samples (~12.5% of the total number of samples) were re-analyzed, and included samples from both genders and samples collected on day 1 and day 91. The re-analysis data for Compound I and M7 were compared with data from the original assay.

3. Results and discussion

3.1. Method development

Upon optimization of the MRM transitions, it was found that both the analytes and the internal standard have the same dominant product ion m/z 334. The chromatographic method was therefore developed to ensure separations of Compound I and M7 to avoid any possibility of interference between the two analytes. Several mobile phases were tested and it was found that a gradient using 5 mM ammonium formate in acetonitrile/water (95/5, v/v) pH 7.0 (mobile phase B) and 5 mM ammonium formate in acetonitrile/water (5/95, v/v) pH 7.0 (mobile phase A) provided the appropriate separation of Compound I and M7 using a reverse phase C_{18} column. As shown in Fig. 2, Compound I and M7 were well separated with retention times of 1.85 and 1.47 min, respectively. There was a small peak observed at 1.87 min in M7 chromatogram which could be due to in-source degradation of Compound I to M7. Since Compound I and M7 were chromatographically separated, there was no impact on the analysis.

3.2. Method validation

3.2.1. Linearity and LLOQ

The slopes, intercepts, and correlation coefficients obtained for the three validation batches are shown in Table 1. The correlation coefficient (r^2) for both Compound I and M7 was greater than 0.99 indicating a good fit of the regression model over the concentration range of 1.00–1000 ng/mL of the calibration curve. An example chromatogram for the LLOQ (1.00 ng/mL) is shown in Fig. 2 showing a good signal-to-noise ratio >200 for both analytes.

3.2.2. Precision and accuracy

Both intra- and inter-day precision and accuracy of the method were evaluated from six replicates of the QC samples at four different concentrations (LLOQ, low, medium and high QC). The experiments were performed on three different days and the data are summarized in Table 2. The intra- and inter-day precision (% CV) and accuracy (% bias) at all concentrations tested were below 15% for Compound I and M7.

3.2.3. Carryover

Carryover was evaluated by determining the peak areas of two control samples run after the injection of the upper limit of quantitation (ULOQ) calibration standard. The peak areas of the control samples were less than 20% of the average peak area of the lower limit of quantitation calibration standards for both Compound I and M7.

3.2.4. Matrix effect and selectivity

The measured concentrations for each lot of plasma at the low QC concentration were compared with the nominal concentration. The % bias in 6 lots of plasma tested was below 6% for both Compound I and M7 indicating that there was no significant matrix effect.

Assay selectivity was evaluated using samples prepared from six different lots of blank plasma and analyzed with and

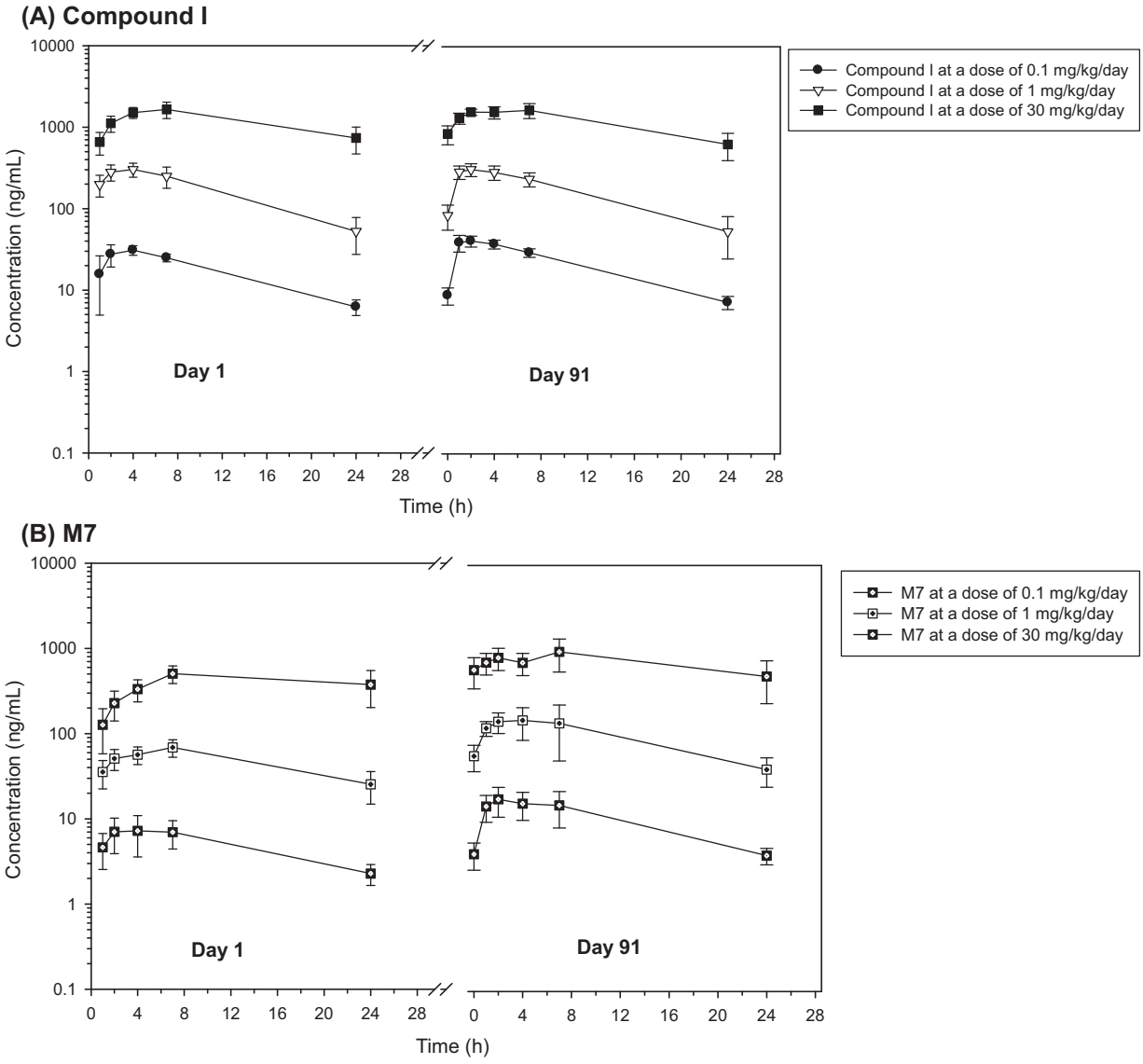

Fig. 3. Mean plasma concentration-time profiles of (A) Compound I and (B) M7 after once daily oral administration of 0.1, 1 or 30 mg/kg of Compound I in Cynomolgus monkeys on day 1 and day 91.

without the internal standards. No interference from endogenous substances was observed at the retention times for each analyte.

3.2.5. Stability

The stability of Compound I and M7 in Cynomolgus monkey plasma under different conditions was studied at the low and high QC concentrations. The results are shown in Table 3. Both compounds were stable for at least 4 h at room temperature with a % bias of less than 2%. The effect of freeze-thaw cycles on the stability of Compound I and M7 was also studied. No significant change (<6%) in the concentrations of either analyte was observed after three freeze-thaw cycles. No significant loss (<7%) of either analyte was observed after storage of the QC samples at −70 °C for 183 days. An analytical batch, including QC and calibration samples, was reanalyzed after storage in the autosampler at 13 °C for 71 h. Results for the reinjected calibration standard and QC samples were all within the acceptance criteria for the assay (e.g. accuracy and precision ≤20% for LLOQ samples and ≤15% for all other samples).

3.2.6. Extraction recoveries

Recoveries were determined by comparing the ratio of the average peak areas of extracted QC samples (i.e. after protein precipitation with working IS) to the average peak areas of extracted blank plasma spiked with equivalent concentrations of Compound I, M7, d_3-Compound I and d_3-M7. The mean recoveries for Compound I, M7, d_3-Compound I, and d_3-M7 were found to be 106%, 107%, 108% and 105%, respectively.

3.2.7. Hemolysis

The low and high QC samples in mid- and high level hemolyzed plasma were studied. All measured concentrations were within ±15% of the nominal concentrations. No matrix effect due to hemolysis was observed.

3.2.8. Application to a toxicokinetic study

Fig. 3 shows the mean (±SD) plasma Compound I and M7 concentration-time profiles on day 1 and day 91 after daily oral administration of 0.1, 1 and 30 mg/kg of Compound I to Cynomolgus monkeys for 91 days. Toxicokinetic parameters are shown in

Table 5
Comparison of mean $AUC_{0-24\,h}$ of M7 to the mean $AUC_{0-24\,h}$ of Compound I.

Dose (mg/kg/day)	Mean $[AUC]_{M7}$/mean $[AUC]_{Compound\ I}$ [a]	
	Day 1	Day 91
0.1	0.286	0.481
1	0.280	0.579
30	0.339	0.609

[a] The mean [AUC] was calculated using both genders ($n = 8$).

Table 4. The mean exposure, based on C_{max} and $AUC_{0-24\,h}$, increased approximately 6- to 11-fold from 0.1 to 1 mg/kg/day on day 1 and day 91 for both genders. The increases in exposures (5- to 10-fold) from 1 to 30 mg/kg/day were less than dose proportional on day 1 and day 91 for both genders. No significant gender differences in exposure were observed for either Compound I or M7. Repeated daily dosing of Compound I for 91 days showed no accumulation of Compound I while an approximately 2-fold accumulation of M7 was observed. The ratio of $AUC_{0-24\,h}$ of M7 to that of Compound I was approximately 0.3 on day 1, and increased to 0.5–0.6 on day 91 as shown in Table 5.

3.2.9. Incurred sample reanalysis

A total of 48 samples were re-analyzed. The % differences between the reassay concentrations and the original concentrations were all less than 20% of their mean values and met the acceptance criteria for incurred sample reanalysis [13].

4. Conclusion

A simple, sensitive, selective and reproducible LC–MS/MS method was developed and validated for the quantitation of a p38 MAP kinase inhibitor (Compound I) and its amide hydrolyzed metabolite (M7) in Cynomolgus Monkey plasma over the concentration range of 1.00–1000 ng/mL. This method was successfully used in the support of a GLP toxicokinetic study.

References

[1] S. Kumar, J. Boehm, J.C. Lee, p38 MAP kinases: key signaling molecules as therapeutic targets for inflammatory diseases, Nat. Rev. Drug Discov. 2 (2003) 717–726.
[2] J. Saklatvala, et al., The p38 MAP kinase pathway as a therapeutic target in inflammatory disease, Curr. Opin. Pharmacol. 4 (2004) 372–377.
[3] J. Lee, et al., A protein kinase involved in the regulation of inflammatory cytokine biosynthesis, Nature 372 (1994) 739–746.
[4] J. Raingeaud, S. Gupta, J.S. Rogers, M. Dickens, J. Han, R.J. Ulevitch, R.J. Davis, Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine, J. Biol. Chem. 270 (1995) 7420–7426.
[5] J.C. Lee, A.M. Badger, D.E. Griswold, D. Dunnington, A. Truneh, B. Votta, J.R. White, P.R. Young, P.E. Bender, Bicyclic imidazoles as a novel class of cytokine biosynthesis inhibitors, Ann. N.Y. Acad. Sci. 696 (1993) 149–170.
[6] H. Hanafusa, J. Ninomiya-Tsuji, N. Masuyama, M. Nishita, J. Fujisawa, H. Shibuya, K. Matsumoto, E. Nishida, Involvement of the p38 mitogen-activated protein kinase pathway in transforming growth factor-β-induced gene expression, J. Biol. Chem. 274 (1999) 27161–27167.
[7] K. Ono, J. Han, The p38 signal transduction pathway: activation and function, Cell Signal. 12 (2000) 1–13.
[8] J.C. Lee, S. Kumar, D.E. Griswold, D.C. Underwood, B.J. Votta, J.L. Adams, Inhibition of p38 MAP kinase as a therapeutic strategy, Immunopharmacology 47 (2000) 185–201.
[9] J. Prakash, V. Saluja, J. Visser, F. Moolenaar, D.K.F. Meijer, K. Poelstra, R.J. Kok, Bioanalysis and pharmacokinetics of the p38 MAPkinase inhibitor SB202190 in rats, J. Chromatogr. B 826 (2005) 220–225.
[10] C. Stambe, R.C. Atkins, G.H. Tesch, T. Masaki, G.F. Schreiner, D.J. Nikolic-Paterson, The role of p38α mitogen-activated protein kinase activation in renal fibrosis, J. Am. Soc. Nephrol. 15 (2004) 370–379.
[11] U.S. FDA, Guidance for Industry, Safety Testing of Drug Metabolites, US Department of Health and Human Services, US FDA, Center for Drug Evaluation and Research, Rockville, MD, USA, 2008.
[12] N.C. Hughes, N. Bajaj, J. Fan, E.Y.K. Wong, Assessing the matrix effects of hemolyzed samples in bioanalysis, Bioanalysis 1 (2009) 1057–1066.
[13] D. Fast, M. Kelley, C. Viswanathan, J. O'Shaughnessy, S. King, A. Chaudhary, R. Weiner, A. DeStefano, D. Tang, Workshop report and follow up-AAPS workshop on current topics in GLP bioanalysis: assay reproducibility for incurred samples-implication of Crystal city recommendations, AAPS J. 11 (2009) 238–241.