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A simple and rapid UHPLC-MS/MS method for the quantitation of the dual aurora kinase A/B inhibitor SCH-1473759 in murine plasma

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Abstract

The Aurora kinase family facilitates cell division through various processes and is overexpressed in a wide variety of human cancers, leading to aneuploidy. For that reason, these enzymes are currently targets of a rising class of anticancer drugs, with some molecules already in therapeutic use. In this study, a new UHPLC-MS/MS method was developed and validated to quantitate a new pan Aurora kinase inhibitor still in preclinical development, SCH-1473759. This bioanalytical method employed a liquid-liquid extraction from plasma using ethyl acetate before evaporation. Calibration range encompassed 0.5–2500 ng/mL. The inter- and intra-day accuracy and precision were assessed over five quality control levels; all within limits required by the FDA guidelines. Assay applicability was demonstrated in a first-in-animals study with Mice oral administration, where the maximum plasma concentration (34 ng/mL) occurred at 1h, the half-life (1h) was consistent with a previous IV study, and oral bioavailability was poor ($F=0.002$).

Keywords

Aurora kinases; UHPLC-MS/MS pharmacokinetics; Mice

1. Introduction

The Aurora kinase family comprises serine/threonine kinases that facilitate cell division through regulation of the key cellular processes of: cell cycle entry, centrosome duplication

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and maturation, mitotic spindle assembly, microtubule-kinetochore attachment, and cytokinesis [1]. Aurora kinase A (AURKA) and B (AURKB) share 70% homology, yet have distinct localization patterns and functions during cell division [2,3]. AURKA localizes to the centrosomes in prophase where it functions to promote cell cycle entry and maturation of the centrosomes and mitotic spindle [4,5]. AURKB is the enzymatically active member of the chromosomal passenger complex and localizes to the centromeres in prophase to regulate microtubule-kinetochore attachments. Both AURKA and AURKB localize to the mid-body region following anaphase to promote cytokinesis [6].

AURKA and AURKB have become desirable targets for therapeutic intervention, as both are frequently overexpressed in a wide range of human cancers that leads to aneuploidy. Several selective and pan-Aurora kinase inhibitors have demonstrated antitumor activity in preclinical studies [7,8]. Alisertib (MLN8237) and barasertib (AZD1152) are known selective AURKA and AURKB inhibitors, respectively, currently being studied clinically for efficacy in a variety of cancers [9–14]. A novel pan-Aurora kinase inhibitor, SCH-1473759 (Fig. 1), with activity against AURKA (IC_{50} 4 nM) and AURKB (IC_{50} 13 nM) [15], is currently in preclinical development. While there was one previous pharmacokinetic (PK) study done via IV injection [15], none provide data for oral bioavailability. Further, there is no published bioanalytical assay available to quantitate SCH-1473759. Therefore, this report presents details of a novel UHPLC-MS/MS assay for measuring SCH-1473759 in murine plasma.

2. Experimental

2.1. Materials

SCH-1473759 and alisertib (the internal standard; IS) were provided by the National Institutes of Health (NIH, Bethesda, MD) repository. Optima-grade acetonitrile (ACN) and methanol were obtained from Fisher Scientific (Pittsburgh, PA). Dimethyl sulfoxide (DMSO) and ethyl acetate were purchased from Sigma-Aldrich (St. Louis, MO). Drug-free heparinized, pooled Balb-C mouse plasma (0.2 μ m filtered) was purchased from Bioreclamation IVT (Baltimore, MD).

2.2. Preparation of stock solutions

Master stock solutions were prepared individually by dissolving SCH-1473759 and alisertib in DMSO at 1 mg/mL concentrations. Serial dilutions (working stocks) were prepared in DMSO from each individual master stock and stored in glass vials at -80°C for the preparation of calibration and quality control (QC) samples. For each analytical run, calibration standards in drug-free mouse plasma were freshly prepared in duplicate at concentrations of 0.5, 5, 50, 100, 250, 500, 1000 and 2500 ng/mL. Quality control (QC) samples were also prepared daily at concentrations of 0.5 (lower limit of quantification; LLOQ), 1.5 (low-range; LQC), 750 (mid-range; MQC), 2000 (high-range; HQC), and 25,000 ng/mL (10-fold dilution; DQC).

2.3. Sample preparation

Frozen standards and samples (50 μ L) were thawed before homogenization by vortex-mixing. A liquid-liquid extraction was performed by adding 1 mL of ethyl acetate containing 20 ng/mL of alisertib (internal standard). This mixture was briefly vortexed, then centrifuged for 10 min at 13,200 rpm in a mini-centrifuge before the supernatant was dried down with desiccated nitrogen gas in a TurboVap96 (Biotage, Charlotte, NC) with a plate temperature of 40°C, then reconstituted with 100 μ L of H₂O/ACN (60/40, v/v).

2.4. Instrument conditions

Ten microliters of each sample were injected onto a Waters ACQUITY UPLC[®] system. Chromatographic separation was achieved on a high strength silica (HSS) pentofluorophenyl (PFP) C18-A, 2.0 $\times$ 50 mm (1.7 μ m particle size), preceded by a HSS PFP C18 guard column (1.7 μ m particle size). The mobile phase consisted of A: 0.1% formic acid in water, and B: 0.1% formic acid in ACN with a flow rate of 0.3 mL/min and a total run time of 7 min running with isocratic A-B (60:40, v/v). The column eluent was directed into a Quattro Premier XE triple quadrupole mass spectrometer. The mass spectrometer monitored SCH-1473759 (m/z 427.2 $\rightarrow$ 270.0) and the internal standard alisertib (m/z 519.0 $\rightarrow$ 328.2) using multiple reaction monitoring (MRM) in the positive ion mode. Universal mass spectrometric settings included capillary voltage of 3.5 kV, cone voltage of 40 V, extractor voltage 3 V, RF Lens 1.0, source temperature of 120°C, desolvation temperature 450°C, cone gas flow 20 L/h, desolvation gas flow 600 L/h, collision energies of 45 eV and 29 eV (argon damping gas) for SCH-1473759 and alisertib, respectively, with dwell times of 200 msec for both analytes. MRM peak integrations and data analyses were performed using the QuanLynx program in MassLynx 4.1.

2.5. Validation

2.5.1. Linearity—Calibration curves for SCH-1473759 were constructed by least-squares linear regression analysis of an eight-point calibration curve (0.5–2500 ng/mL) by plotting the ratio of the analyte peak area to the IS peak area, using $1/x^2$ as a weighting factor, where x is the ratio of the nominal analyte:IS concentration. Calibrator response functions and choice of regression analysis were investigated by calculating correlation coefficients (r^2) and the percent closeness of the mean observed concentration relative to the nominal (% DEV) for all calibrators. The lower limit of quantitation (LLOQ) was defined as the lowest calibration standard capable of producing a peak with a signal-to-noise (S/N) ratio of 5.

2.5.2. Accuracy and precision—Quality control (QC) standards of SCH-1473759 prepared at five levels (LLOQ, LQC, MQC, HQC, and DQC) were used to assess assay precision and accuracy. Each QC level was analyzed in quintuplet and prepared fresh on each of four different days. Each run consisted of blank plasma samples, internal standard only, and calibration standards in duplicate; QC samples in quintuplets ($n=20$ total). The repeatability of the assay was determined by the within-run precision (WRP) and between-run (BRP), as described previously [16]. FDA guidelines for bioanalytical validation were followed, with $\pm 15\%$ variability in accuracy and precision allowed except for the LLOQ, where $\pm 20\%$ variability is acceptable [17].

2.5.3. Stability—The stability of SCH-1473759 in mouse plasma at physiological temperature was assessed over a 2h period at both a low (1.5 ng/mL) and high (2000 ng/mL) concentration. Samples at both concentrations were either extracted immediately or incubated at 37°C in plasma for 2 h before extraction, each in triplicate. The analyte concentration after 2h was compared to the concentration of freshly prepared samples in the same analytical run.

Stability tests were performed to examine the potential for degradation of SCH-1473759 in mouse plasma during three freeze/thaw cycles. Samples were assayed at two concentrations (1.5, 2000 ng/mL) in triplicate. The samples were subjected to three freeze/thaw cycles at –80°C, with each freeze cycle lasting at least 12 h. The analyte concentration after each storage period was compared to the concentration of freshly prepared samples in the same analytical run.

The post-preparative stability of SCH-1473759 and alisertib in the sample plates (96-well collection plates) pending analysis in the autosampler was assessed. Samples were reinjected and reanalyzed 24 h after the initial analysis and compared to the original values obtained from those same samples prepared 24h prior.

2.5.4. Extraction efficiency—The extraction efficiency, or recovery, of the liquid-liquid extraction was assessed by comparing analyte peak areas from both a low (1.5 ng/mL; n=5) and high (2000 ng/mL; n=5) concentration, both before and after the addition of the liquid extraction solvent. Matrix effects were assessed by the percent difference in the slope of the calibration curve from standards spiked into reconstitution solution vs slope of standards spiked into mouse plasma.

2.6. Preclinical application

To demonstrate the applicability of this method for measuring SCH-1473759 in preclinical studies, healthy 6 week-old female SCID-Beige (strain 250) mice were administered 20 mg/kg SCH-1473759 *via* oral gavage. SCID mice were chosen due to their common use in xenograft studies. The formulation chosen (20% 2-hydroxypropyl-beta-cyclodextrin) was based upon the most recent publications of xenograft studies using alisertib [18] and intraperitoneal injection of SCH-1473759 [15].

Following dosing, blood (~200uL) was drawn via mandibular bleed into heparinized tubes at the following time points (in duplicate mice) post-dose: 0.25, 0.5, 1, 2, 4, 7, 10, and 24h (n=18 animals). Mice were not subjected to more than one blood collection for the study. Blood was centrifuged at 1200 × g for 5 min and plasma frozen at –80°C until time of bioanalysis. This was the first *in vivo* oral administration of this compound. Area under the curve (AUC) was calculated using Bailer's method for destructive sampling, with standard error [19]. The NCI Animal Care and Usage Committee approved this animal protocol (PB-035) and all animals were handled and treated according to ethical guidelines.

3. Results and discussion

3.1. Selectivity

The retention times of SCH-1473759 and alisertib, were 2.91 and 5.75 min, respectively (Fig. 2). The use of UHPLC provided sharp, symmetrical peaks that were well-resolved and separated from interfering matrix peaks that were eluted following an organic solvent gradient to cleanse the column.

3.2. Linearity, accuracy, and precision

The calibration standards, ranging from 0.5–2500 ng/mL, were run in duplicate on each of four days (n=8). The eight calibration standards proved accurate and precise, with a mean ($\pm$ SD) calibration curve linear correlation (r^2) of 0.9953 ± 0.0013 (n=4). Four quality control (QC) samples, at low, mid, high, and 10-fold diluted concentrations, plus LLOQ, were run in quintuplet daily over four days. All QC levels proved accurate and precise, within the required 15% (20% for LLOQ samples) (Table 1).

3.3. Stability

SCH-1473759 demonstrated sufficient stability in plasma incubated at 37°C through 2h, with <9% difference in measured concentrations from freshly extracted samples at both low and high concentrations. Three freeze/thaw cycles had a negligible impact (<8%) on SCH-1473759 stability. The 24h post-preparative measurements were within acceptable limits (<15% change) at the mid and high level, except at the low end, where sample evaporation led to an average of 32% increase in measured concentration. This suggests incurred sample reanalysis may not be accurate.

3.4. Extraction efficiency/matrix effects

The mean overall recoveries for SCH-1473759, estimated by comparing the mass spectrometric signal response of the analyte spiked into mouse plasma before extraction *versus* analyte spiked after extraction at both a low (1.5 ng/mL) and a high (2000 ng/mL) concentration, were approximately 85% and 95%, respectively. Although there were significant matrix effects, resulting in 75–78% lower signal vs clean solvent, the instrument sensitivity, and excellent extraction recovery, allowed for a LLOQ of 0.5 ng/mL.

3.5. Clinical application

This UHPLC-MS/MS assay was able to selectively quantitate SCH-1473759 in mouse plasma following *in vivo* oral administration. Following a 20 mg/kg dose given orally, absorption was quite poor (C_{MAX} =34 ng/mL), likely due to improper formulation. Measured plasma concentrations were above the LLOQ at 7h post dose, but not beyond (Fig. 3). SCH-1473759 peak concentrations occurred at 1h before a monophasic elimination, with a half-life of roughly 1.2h(70.8 min), which was consistent with an IV study in mice [15]. Comparing oral exposure from this study ($0.1289 \pm 0.02h^* uM$) with exposure following IV injection of 2.5 mg/kg ($0.90 h^* uM$) gave an estimate of bioavailability as $F=0.002$.

4. Conclusions

In conclusion, this simple, sensitive, robust, and reproducible assay for the quantitation of the dual Aurora kinase A/B inhibitor SCH-1473759 was accurate and precise, and the calibration range was sufficient to monitor plasma levels following a 20 mg/kg dose given to mice orally. The calibration upper limit of 2500 ng/mL was established as the highest concentration within a linear dynamic range based on plasma concentrations achieved following IV injection at 2.5 mg/kg using an 8-fold lower dose than this study [15]. Although plasma concentrations in this oral study were lower than expected, future studies aim to improve formulation, hence absorption and bioavailability.

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Abbreviations

UHPLC	ultra-high performance liquid chromatography
MS	mass spectrometry
MS/MS	tandem mass spectrometry
QC	quality control
LLOQ	lower limit of quantification
ACN	acetonitrile
%DEV	percent deviation from the nominal value
WRP	within-run precision
BRP	between-run precision

References

- [1]. Mountzios G, Terpos E, Dimopoulos MA, Aurora kinases as targets for cancer therapy, *Cancer Treat. Rev* 34 (2008) 175–182. [PubMed: 18023292]
- [2]. Adams RR, Carmena M, Earnshaw WC, Chromosomal passengers and the (aurora) ABCs of mitosis, *Trends Cell Biol* 11 (2001) 49–54. [PubMed: 11166196]
- [3]. Nigg EA, Mitotic kinases as regulators of cell division and its checkpoints, *Nat. Rev. Mol. Cell Biol* 2 (2001) 21–32. [PubMed: 11413462]
- [4]. Barr AR, Gergely F, Aurora-A: the maker and breaker of spindle poles, *J. Cell Sci* 120 (2007) 2987–2996. [PubMed: 17715155]
- [5]. Vader G, Lens SM, The Aurora kinase family in cell division and cancer, *Biochim. Biophys. Acta* 1786 (2008) 60–72. [PubMed: 18662747]
- [6]. Ruchaud S, Carmena M, Earnshaw WC, Chromosomal passengers: conducting cell division, *Nat. Rev. Mol. Cell Biol* 8 (2007) 798–812. [PubMed: 17848966]
- [7]. Manfredi MG, Ecsedy JA, Meetze KA, Balani SK, Burenkova O, Chen W, et al., Antitumor activity of MLN8054, an orally active small-molecule inhibitor of Aurora A kinase, *Proc. Natl. Acad. Sci. U. S. A* 104 (2007) 4106–4111. [PubMed: 17360485]

- [8]. Wilkinson RW, Odedra R, Heaton SP, Wedge SR, Keen NJ, Crafter C, et al., AZD1152 a selective inhibitor of Aurora B kinase, inhibits human tumor xenograft growth by inducing apoptosis, *Clin. Cancer Res* 13 (2007) 3682–3688. [PubMed: 17575233]
- [9]. Barr PM, Li H, Spier C, Mahadevan D, LeBlanc M, Ul Haq M, et al., Phase II intergroup trial of alisertib in relapsed and refractory peripheral T-cell lymphoma and transformed mycosis fungoides: SWOG 1108, *J. Clin. Oncol* 33 2399–2404.
- [10]. Boss DS, Witteveen PO, van der Sar J, Lolkema MP, Voest EE, Stockman PK, et al., Clinical evaluation of AZD1152 an iv. inhibitor of Aurora B kinase, in patients with solid malignant tumors, *Ann. Oncol* 22 (2011) 431–437. [PubMed: 20924078]
- [11]. Friedberg JW, Mahadevan D, Cebula E, Persky D, Lossos I, Agarwal AB, et al., Phase II study of alisertib a selective Aurora A kinase inhibitor, in relapsed and refractory aggressive B-and T-cell non-Hodgkin lymphomas, *J. Clin. Oncol* 32 (2014) 44–50. [PubMed: 24043741]
- [12]. Goldberg SL, Fenaux P, Craig MD, Gyan E, Lister J, Kassis J, et al., An exploratory phase 2 study of investigational Aurora A kinase inhibitor alisertib (MLN8237) in acute myelogenous leukemia and myelodysplastic syndromes, *Leuk. Res. Rep* 3 (2014) 58–61. [PubMed: 25068104]
- [13]. Schwartz GK, Carvajal RD, Midgley R, Rodig SJ, Stockman PK, Ataman O, et al., Phase I study of barasertib (AZD1152) a selective inhibitor of Aurora B kinase, in patients with advanced solid tumors, *Invest. New Drugs* 31 (2013) 370–380. [PubMed: 22661287]
- [14]. Wetmore C, Boyett J, Li S, Lin T, Bendel A, Gajjar A, et al., Alisertib is active as single agent in recurrent atypical teratoid rhabdoid tumors in 4 children, *Neuro. Oncol* 17 (2015) 882–888. [PubMed: 25688119]
- [15]. Yu T, Tagat JR, Kerekes AD, Doll RJ, Zhang Y, Xiao Y, et al., Discovery of a potent injectable inhibitor of Aurora kinases based on the imidazo-[1,2-a]-pyrazine core, *ACS Med. Chem. Lett* 1 (2010) 214–218. [PubMed: 24900197]
- [16]. Peer CJ, Spencer SD, VanDenBerg DA, Pacanowski MA, Horenstein RB, Figg WD, A sensitive and rapid ultra HPLC-MS/MS method for the simultaneous detection of clopidogrel and its derivatized active thiol metabolite in human plasma, *J. Chromatogr. B Anal. Technol. Biomed. Life Sci* 880 (2012) 132–139.
- [17]. US Food and Drug Administration. Guidance for Industry: Analytical Procedures and Methods Validation for Drugs and Biologics (2015).
- [18]. Palani S, Patel M, Huck J, Zhang M, Balani SK, Yang J, et al., Preclinical pharmacokinetic/pharmacodynamic/efficacy relationships for alisertib, an investigational small-molecule inhibitor of Aurora A kinase, *Cancer Chemother. Pharmacol* 72 (2013) 1255–1264. [PubMed: 24101146]
- [19]. Bailer AJ, Testing for the equality of area under the curves when using destructive measurement techniques, *J. Pharmacokinet. Biopharm* 16 (1988) 303–309. [PubMed: 3221328]

SCH-1473759

MW 426.5 g/mol

Alisertib (IS)

MW 518.9 g/mol

Fig. 1.

Molecular structures of SCH-1473579 and the internal standard alisertib.

Fig. 3.
Mean log-transformed SCH-1473759 plasma concentration vs time curve.

Table 1

Accuracy and Precision.

Nominal (ng/ml)	Gm (ng/ml)	SD(ng/ml)	DEV (%)	WRP (%)	BRP (%)	n
0.5 (LLQQ)	0.53	0.05	96.3	7.9	6.5	17
1.5 (LQC)	1.66	0.24	110	14.0	-	17
750 (MQC)	728	54	97.1	7.6	-	20
2000 (HQC)	1810	123	90.5	4.2	6.0	20
25,000 (DQC)	25,150	2890	99.4	9.9	6.4	20

Abbreviations: GM, grand mean; SD, standard deviation; DEV (%) relative deviation from nominal value; WRP, within-run precision; *n*, number of replicate observations within each validation run.