Journal of Chromatography B, 901 (2012) 85–92

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

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

Determination of pazopanib (GW-786034) in mouse plasma and brain tissue by liquid chromatography–tandem mass spectrometry (LC/MS–MS)

Mukul Minocha, Varun Khurana, Ashim K. Mitra*

University of Missouri-Kansas City, School of Pharmacy, Division of Pharmaceutical Sciences, USA

ARTICLE INFO

Article history:
Received 1 November 2011
Accepted 6 June 2012
Available online 15 June 2012

Keywords:
Mouse plasma
Pazopanib
GW-786034
Pharmacokinetics
LC/MS–MS
Brain tissue

ABSTRACT

A simple, rapid and sensitive liquid chromatography–tandem mass spectrometric (LC/MS–MS) method has been developed and validated for the quantitative determination of pazopanib in mouse plasma and brain tissue homogenate. Single liquid–liquid extraction step with ethyl acetate was employed for analysis of pazopanib and the internal standard (IS); vandetanib. HPLC separation was performed on an XTerra® MS C18 column 50 mm × 4.6 mm, 5.0 μm. The mobile phase consisted of 70% acetonitrile and 30% water with 0.1% formic acid, pumped at a flow rate of 0.25 ml/min. Analysis time was 3.5 min per run and both the analyte and IS eluted within 1.8–2.0 min. Multiple reactions monitoring (MRM) mode was utilized to detect the compounds of interest. The mass spectrometer was operated in the positive ion mode for detection. The precursor to product ions (Q1→Q3) selected for pazopanib and internal standard during quantitative optimization were (*m*/*z*) 438.1→357.2 and 475.0→112.2 respectively. The calibration curves were linear over the range of 3.9–1000 ng/ml in both biological matrices. Lower limit of quantification (LLOQ) for mouse plasma and brain tissue was 3.9 ng/ml. The values for inter and intra day precision and accuracy were well within the ranges acceptable for analytical assessment (<15%). This method was applied to determine brain to plasma concentration ratio and relevant pharmacokinetic parameters of pazopanib after a single intravenous dose of 5 mg/kg in FVB wild type mice.

1. Introduction

Angiogenesis plays a pivotal role in tumor growth and helps sustain metastasis from primary tumor cells [1]. Vascular endothelial growth factor receptor (VEGF) is considered to be a major determinant in pathogenesis of several tumor types [2–4]. Apart from VEGF, tumor cell proliferation is also dependent on other growth factors such as platelet derived growth factor (PDGF) [5] and epidermal growth factor (EGF) [6]. These growth factors are dysregulated in numerous cancer types and hence, targeting these receptors is an attractive strategy. Pazopanib (GW-786034, Votrient®) is an orally active multitargeted tyrosine kinase inhibitor (TKI) that targets VEGFR-1, -2, and -3, PDGFR-α, PDGFR-β, and c-Kit. It acts by competing with the binding of ATP to the intracellular tyrosine kinase domain of growth factor receptors, thereby inhibiting receptor autophosphorylation and blocking downstream signal transduction [7]. Numerous novel small molecule TKIs are under development and have already been approved by the United States Food and Drug Administration (USFDA) for the treatment of solid tumors.

Preclinical studies suggest an IC_{50} value of pazopanib for human VEGF-2 inhibition in low nanomolar range [8]. Potent antiangiogenic and antitumor activity of pazopanib has been reported in several human tumor xenografts including colon, melanoma, prostate, renal, breast and lung [8]. Furthermore, the steady-state concentration of pazopanib determined from preclinical studies showed a strong correlation with the pharmacodynamic effects and antitumor activity in a Phase I clinical trial [8]. Recently, USFDA approved pazopanib for the treatment of advanced renal cell carcinoma [9]. Currently, many clinical trials are ongoing for testing the efficacy of pazopanib alone and in combination with other anti-cancer drugs for the treatment of metastatic cervical cancer, breast cancer, corneal neovasularization and recurrent brain tumors (www.clinicaltrials.gov). However, the tight endothelial junctions located in brain capillaries which are further fortified by the presence of ABC efflux transporters (blood–brain barrier, BBB) can restrict the brain penetration of pazopanib and may lead to its therapeutic inefficacy when used as an anti-tumor agent for recurrent brain tumors.

To systematically examine the preclinical plasma pharmacokinetics and brain accumulation of pazopanib in a reproducible and precise manner, a sensitive and validated assay is necessary. To the best of our knowledge, there is no published report in the

* Corresponding author at: Division of Pharmaceutical Sciences, School of Pharmacy, University of Missouri-Kansas City, 2464 Charlotte Street, Kansas City, MO 64108, USA. Tel.: +1 816 235 1615; fax: +1 816 235 5190.
E-mail address: mitraa@umkc.edu (A.K. Mitra).

1570-0232/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jchromb.2012.06.004

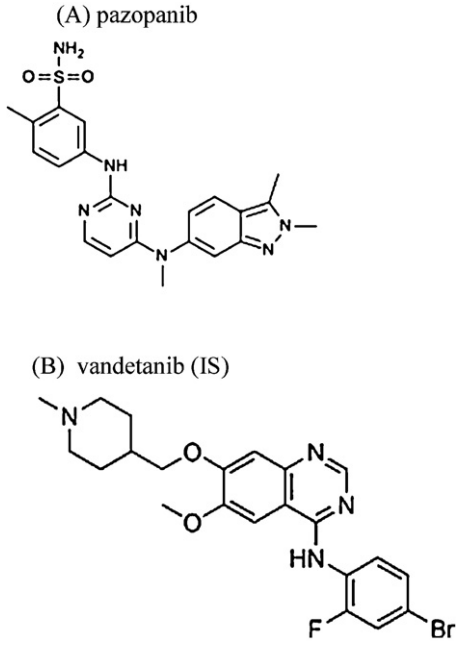

Fig. 1. Chemical structures of pazopanib and internal standard, vandetanib.

literature that demonstrates validation of a sensitive assay for the determination of pazopanib in biological tissues. Hence, in this paper, we describe a simple liquid–liquid extraction method that utilizes reverse phase liquid chromatography coupled with mass spectrometry (LC/MS–MS) technique. This method requires just 100 μl aliquots of plasma and brain tissue homogenate to quantify pazopanib in preclinical pharmacokinetic and brain distribution studies by employing vandetanib (a TKI) as an internal standard (Fig. 1b)

2. Materials and methods

2.1. Chemicals

Pazopanib and internal standard (IS) vandetanib were purchased from LC labs (Woburn, MA). Drug free mouse plasma was obtained from Valley Biomedicals (Winchester, VA). Formic acid and HPLC grade water and acetonitrile were procured from Fisher Scientific (Pittsburgh, PA). All other reagents were HPLC or reagent grade and were purchased from Sigma–Aldrich (St. Louis, MO).

2.2. Stock solution, calibration standards and quality controls

Stock solution of pazopanib and IS were prepared in methanol and diluted to a final concentration of 100 μg/ml. All stock solutions were stored in $-80 \pm 5\,^{\circ}C$. A set of nine non-zero calibration standards ranging from 3.9 ng/ml to 1000 ng/ml were prepared by spiking the drug free mouse plasma and brain homogenate samples with appropriate amounts of pazopanib. The quality control (QC) samples 3.9 ng/ml (LLOQ), 7.81 ng/ml (LQC), 125 ng/ml (MQC) and 1000 ng/ml (HQC) were prepared in a similar manner and were included in non-zero calibration standards. All calibration standards were prepared in triplicate and quality control samples were prepared in six replicates.

2.3. Sample preparation

A 100 μl aliquot each of mouse plasma and brain homogenate (prepared by adding 3 vol. of 5% bovine serum albumin in water) were transferred into a 1.5 ml polypropylene microcentrifuge tubes. To this aliquot, 40 ng of IS, vandetanib solution (40 μl from working solution of 1 μg/ml in methanol) was added and mixture was vortexed for 10 s. Next, 900 μl of ice cold ethyl acetate was added and the mixture was vortexed vigorously for 2 min followed by centrifugation at 10,000 rpm for 7 min at 4 °C to cause efficient separation of aqueous and organic layer. Seven hundred microliters of the organic layer was removed and transferred to freshly labeled microcentrifuge tubes and dried in vacuum (Gene-vac DD-4X). The dried residue was reconstituted in 100 μl of mobile phase (70:30:0.1, acetonitrile:water:formic acid, in %v/v) and 10 μl was injected into the LC/MS–MS for analysis.

2.4. Chromatographic and mass spectrometric conditions

The chromatographic system consisted of Shimadzu quaternary pump, vacuum degasser and autosampler (Shimadzu scientific instruments, Columbia MD, USA) coupled to QTrap® API-3200 mass spectrometer (Applied Biosystems, Foster City, CA, USA). HPLC separation was performed on an XTerra® MS C_{18} column 50 mm × 4.6 mm, 5.0 μm (Waters, Milford, MA). The mobile phase consisted of 70% acetonitrile and 30% water with 0.1% formic acid, pumped at a flow rate of 0.25 ml/min. Analysis time was 3.5 min per run.

API-3200 mass spectrometer (Applied Biosystems) equipped with ion spray source was employed for obtaining mass spectra. Data acquisition was carried out by analyst 1.2 software. Multiple reactions monitoring (MRM) mode was utilized to detect the compounds of interest. The mass spectrometer was operated in the positive ion mode for detection. The precursor to product ions (Q1→Q3) selected for pazopanib and vandetanib during quantitative optimization were (*m*/*z*) 438.2→357.2 and 475.0→112.2 respectively. The operational parameters for the tandem mass spectrum of each analyte were obtained after running them in quantitative optimization mode. The turbo ion spray setting and collision gas pressure were optimized (IS voltage: ±5500 V, temperature: 350 °C, nebulizer gas: 30 psi, curtain gas: 30 psi). Peak area ratios of pazopanib and IS were calculated for preparing calibration curves by employing least squares regression analysis and uniform weighting. Parameters obtained from these calibration curves were used for back-calculating pazopanib concentration in mouse plasma and brain homogenate QC samples.

2.5. Method validation

2.5.1. Inter-assay and intra assay variability

Method validation batches for pazopanib quantification in mouse plasma and brain homogenate were performed on three separate occasions. Precision and accuracy batches comprised of three replicates of nine non-zero concentrations of calibration standards and six replicates of QC samples at four different concentrations. Inter-assay and intra assay variability were determined by computing percentage relative error (%RE) and percentage coefficient of variation (%CV).

2.5.2. Limit of quantification

Lower limit of quantification (LLOQ, i.e., the lower calibration level) sample was designated based on the criteria that the variability in accuracy and precision (%CV) was less than 20% with corresponding signal/noise ratio greater than 10, as stated in the FDA guidance for bioanalytical method validation. Signal/noise ratio was calculated based on the peak areas of LLOQ samples verses that of the background noise in true blank samples in respective biological matrices.