Simple Determination of Plasma Ponatinib Concentration Using HPLC

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Ponatinib, a novel tyrosine kinase inhibitor marketed in 2016, is a key drug used for treating chronic myeloid leukemia and Philadelphia chromosome-positive acute lymphoblastic leukemia. This study aimed to develop a simple method for determining plasma ponatinib concentration. The analysis required extraction of a 400-µL sample of plasma and precipitation of proteins using an Oasis HLB cartridge. Ponatinib and bosutinib, which is used as an internal standard, were separated by HPLC using a mobile phase of acetonitrile:0.037 mol/L KH2PO4 (pH 4.5) (39:61, v/v) on a Capcell Pack C18 MG II (25×4.6 mm) monitored at 250 nm, with a flow rate of 1.0 mL/min. This assay method was then used for determining plasma ponatinib concentration in a 42-year-old man treated with ponatinib at 15 mg/d. The calibration curve was found to be linear for the plasma concentration range of 5–250 ng/mL with a regression coefficient ($r^2$) of 0.9999. The coefficients of intra-day and inter-day validation under these concentrations were 2.1–6.0 and 4.5–8.0%, respectively. The assay accuracy was −1.5–9.0%, and the recovery was greater than 86%. The plasma concentration of the patient at 2.5 and 3 h after 15 mg ponatinib administration was 43.6 and 49.3 ng/mL, respectively. This method of HPLC equipped with UV detection for determining plasma ponatinib concentration has several advantages, such as simplicity and applicability to routine therapeutic drug monitoring at hospital laboratories.

Key words ponatinib; bosutinib; HPLC equipped with UV detection method; human plasma concentration
Kyoto, Japan), a UV detector (SPD-20A, Shimadzu), and an auto-sampler (SIL-20AC HT, Shimadzu). The octadecylsilyl column (Capcell Pack C18 MG II, 250 × 4.6 mm i.d., 5 µm, Shiseido, Tokyo, Japan) with a guard column (Capcell Pack C18 MG II guard column, 10 × 4.0 mm, Shiseido) as an analytical column was maintained at 40°C. The detection wavelength was set at 250 nm. The mobile phase consisted of acetonitrile:0.037 mol/L KH₂PO₄ (pH 4.5) (39:61, v/v) and the flow rate was set at 1.0 mL/min.

**Assay Procedure** Ponatinib-spiked plasma (400 µL), with 10 µL international standard (IS) solution (50 ng/mL bosutinib) and 600 µL water, was vortex-mixed for 1 min and applied to the pre-conditioned Oasis HLB cartridges. Subsequently, the cartridges were washed with 1 mL water and 60% methanol in water (v/v). The analytes were then eluted with 1 mL methanol and evaporated to dryness under vacuum at 80°C using a rotary evaporator. The dried residues were reconstituted in 50 µL methanol and a 30-µL aliquot was injected into the HPLC system.

**Calibration and Validation** Ponatinib and bosutinib stock solutions were prepared at concentrations of 1 mg/mL in methanol. Control plasma samples spiked with ponatinib at concentrations of 5, 10, 25, 50, and 250 ng/mL were used to construct the calibration curve. The recovery and accuracy of ponatinib were examined at the same concentrations (n = 5). To confirm assay precision, five sets of control samples were assayed on the same day (intra-day) and on five different days (inter-day) at the same concentrations.

**Sample Stability** The stability of ponatinib at four different concentrations (5, 25, 50, 250 ng/mL) in plasma samples was evaluated. Bench-top stability was tested in samples kept at room temperature for 6 h (n = 5). Processed sample stability was tested following 24 h storage at 4°C (n = 5). Long-term stability was tested after 1 or 2 weeks at −20°C (n = 5). Freeze and thaw stability was tested in samples retrieved from −20°C after three freeze-thaw cycles (n = 5). In addition, the stability of ponatinib (25 ng/mL) in plasma samples was evaluated at −20°C for up to 1 month (n = 5).

**RESULTS**

**Assay Validation and Stability** A typical chromatogram for the plasma ponatinib determination (50 and 5.0 ng/mL) is shown in Figs. 2a and b, respectively. The retention time of ponatinib and the IS was 14 and 8 min, respectively. Interfering peaks were not observed for either ponatinib or the IS (Fig. 2c). A five-point standard calibration curve of ponatinib in the plasma was linear within the range of 5–250 ng/mL. The equation of the calibration curve was

\[ y = 0.0072x - 0.0048 \quad (r^2 = 0.9999) \] (Fig. 3), where \( y \) is the peak height ratio of ponatinib and \( x \) is the plasma concentration of ponatinib (ng/mL).

The lower limit of quantification (LOQ) for ponatinib was 5 ng/mL. The recovery of ponatinib between 5 and 250 ng/mL was greater than 86%. The coefficients of variation (CVs) for intra-day and inter-day at these concentrations were in the range of 2.1–6.0 and 4.5–8.0%, respectively (Table 1). Stability assessments of ponatinib in plasma under various conditions were examined (Table 2). No significant degradation of ponatinib was observed, and the final concentration of ponatinib was within 93.8–105.4% of the theoretical values. In addition, sample stability at a concentration of 25 ng/mL (n = 5) was 97.1% after 1 month of storage as a stock solution at −20°C.

**Application in a Patient Treated with Ponatinib** A 42-year-old man was treated with first-line imatinib (400 mg/d) after diagnosis of CML, followed by dasatinib (100 mg/d) after detection of a T315I mutation in Sep. 2009. Thereafter, he underwent unrelated cord blood transplantation in Jan. 2011.

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**Fig. 2. Typical Chromatograms for the Determination of Plasma Ponatinib**

a. spiked plasma sample containing ponatinib 50 ng/mL. b. spiked plasma sample containing ponatinib 5 ng/mL (LOQ). c. blank plasma sample.
but relapsed. He was then enrolled in a ponatinib clinical trial, and started on 45 mg/d in Feb. 2013. In Nov. 2013, the dosage was reduced to 30 mg/d according to instructions in response to observed accumulation of arterial occlusive events with longer follow-up across the ponatinib clinical program. In Sep. 2014, he was started on amlodipine (5 mg/d) after candesartan (4 mg/d) for treatment of hypertension. In Mar. 2017, the ponatinib dose was reduced from 30 to 15 mg/d, but hypertension remained. The steady state concentrations at 2.5–3 h after ponatinib intake on days 57 and 85 after dose reduction to 15 mg/d were 43.6 ng/mL (Fig. 4) and 49.3 ng/mL, respectively. During these periods, co-administration of amlodipine (10 mg/d), candesartan (8 mg/d), and rosuvastatin (5 mg/d) was continued, and laboratory data were unchanged. Although hypertension was potentially related to ponatinib, this event was under control, and the patient showed a durable complete molecular response (Fig. 5).

**DISCUSSION**

In this study, we developed a sufficiently sensitive method for determining ponatinib plasma concentrations using a simple instrument in the clinical setting. This method using HPLC-UV is applicable for therapeutic drug monitoring of ponatinib-treated patients diagnosed with rare CML.

In our method, the intra-assay and inter-assay variation (under 8.0% at each concentration), accuracy (under 9% at each concentration), and stability under various conditions (under 13% at each concentration) were within the Food and Drug Administration (FDA) guideline recommendations (Tables 1, 2). The range of ponatinib plasma concentration at the therapeutic dosage levels of 15, 30, and 45 mg daily was within the range of 14.7–110.7 ng/mL. Therefore, our method, which functions in the range 5–250 ng/mL ($r^2=0.999$), was suitable for the therapeutic drug monitoring of ponatinib in clinical settings (Fig. 2).
The LOQ of our method was 5 ng/mL for ponatinib (Table 1, Fig. 2). This value was higher than that obtained using LC-MS/MS (LOQ: 0.5 ng/mL). However, other methods using LC-MS/MS were comparable to our method (LOQ: 5 ng/mL). Although our method had a 4–5 min longer run time (15 min) than that of other LC-MS/MS methods, this HPLC-UV method has the advantage of a lower initial cost.

In a CML patient, the ponatinib concentration at 2.5 and 3 h after oral intake of 15 mg of ponatinib was 43.6 and 49.3 ng/mL, respectively (Figs. 4, 5). This was similar to the results of a Japanese phase 1/2 study, in which the mean steady state maximum plasma concentration (C_max: 4 h) and the trough concentration at 15 mg/d were 44.2 and 25.6 ng/mL, respectively. The target trough concentration in the Ponatinib Ph-positive acute lymphoblastic leukemia [ALL] and CML Evaluation (PACE) study was observed to be 40 nM (23 ng/mL), which achieved complete suppression of BCR-ABL mutations in vitro. Although our patient showed controllable grade 3 hypertension as an adverse event (Fig. 5), the plasma concentration of ponatinib was almost within the therapeutic range. The relation between hypertension and the plasma concentration of ponatinib may be useful for future studies.

Metabolites of ponatinib and other drugs, such as anti-hypertension drugs, were not confirmed in our method and caused no interference in the ponatinib and IS chromatogram of our patient. However, most of the anti-hypertension drugs had absorption maxima between 220–260 nm, and the concomitant presence of these drugs in patients treated with ponatinib needs to be checked by observing the interference of their peaks.

Patients treated with ponatinib are currently rare. However, in the near future, in adequate clinical settings, this simple method may be necessary for patients using ponatinib.

CONCLUSION

In conclusion, the reported HPLC-UV method to determine plasma ponatinib concentration has (1) lower initial cost to initiate ponatinib monitoring at the hospital and (2) a sufficient level of LOQ in clinical settings. Our method for determining ponatinib concentration is simple and applicable to routine therapeutic drug monitoring at hospital laboratories.

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Conflict of Interest A.T. received research Grants from Otsuka Pharmaceutical Co., Ltd. The other authors declare no conflict of interest.

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