Pharmacokinetics study of potential anti-CML drug Cyclobentinib (CB1107) by HPLC–MS/MS

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Abstract: Purpose: A simple, sensitive and specific HPLC–MS/MS method was established to analyse the pharmacokinetics of CB1107 in mice. Methods: A simple, selective, and sensitive high-throughput liquid chromatography-tandem mass spectrometry (LC-MS-MS) method has been developed and validated for quantitative determination of CB1107 in rat serum. Chromatographic separation was achieved on a Zorbax Extend C18 Rapid Resolution HD column (4.6 mm × 50 mm, 1.8 µm). The column temperature was maintained at 35°C and at flow rate of 0.6 mL/min. Injection volume was 20 µL. The mobile phases consisted of 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B), and total run time was 30 min. MS-MS detection was performed in the selected monitoring mode of electrospray positive ionization reaction. Results: The pharmacokinetic characteristics of CB1107 in mice belong to the two-compartment model. When the doses were 400 mg/kg, 600 mg/kg, and 800 mg/kg, corresponding area under the plasma concentration-time curve (AUC) respectively were 20.011 ± 2.19 mg/h/L, 26.778 ± 2.19 mg/h/L and 38.82 ± 1.44 mg/h/L, suggesting that CB1107 have a good absorption in the body. And the AUC of three doses are proportional, indicating that CB1107 conforms to linear pharmacokinetics in vivo. Conclusion: This method was successfully applied to study the pharmacokinetics at three different doses of CB1107 after oral administration in mice. In this study, the bioactivity mechanism of CB1107, by the pharmacokinetic investigation of CB1107 in vivo.

Keywords: chronic myelogenous leukemia, Cyclobentinib (CB1107), pharmacokinetics

1 Introduction

Chronic myelogenous leukemia (CML) is a bone marrow hematopoietic stem cell malignant cloning disease, caused by the oncogenic BCR-ABL fusion protein. The signature genetic abnormality of CML is t(9;22) (q34;Q11) translocation, which called the Philadelphia chromosome [1]. The emergence of bcr-abl tyrosine kinase inhibitors (TKIs) extended the life of patients. At present tyrosine kinase inhibitors are still the main drugs used to treat chronic myelogenous leukemia [2].

Imatinib is a specific potent and efficient tyrosine kinase inhibitors (TKI), or the signal transduction inhibitors (signal transduction inhibitors, STI). The mechanism is competitively bind with ATP binding site of the catalytic site of tyrosine kinase, which makes the kinase unable to bind to ATP and lose catalytic activity, thus achieving the effect of targeted therapy on CML [3–6]. Studies have shown that Imatinib can also be used in the treatment of gastrointestinal stromal tumors (GIST) [7,8]. Gastrointestinal stromal tumor (GISTs) is a common of human gastrointestinal mesenchymal tumors, the pathogenesis is functional mutation of proto-oncogene c-Kit. But at present, tumor cells are resistant to imatinib [9]. The main mechanism of CML drug resistance is the point mutations in the ABL kinase domain, among which the T315I mutation is due to the mutation of cytosine at position 944 in the abl gene sequence to thymine (C > T), lead to that the expressed Abl protein was replaced by isoelucine (I) at threonine (T) which at position 315 [10–12]. This substitution directly results in the loss of the oxygen atoms (hydrogen bond donors) needed to form critical hydrogen bonds between Imatinib and abl kinase active domains, resulting in the inability to form hydrogen bonds, thereby weakening Imatinib’s affinity with the kinase active domain [13,14].

CB1107 is a tyrosine kinase inhibitor targeting the mutant strain of imatinib T315I, with novel compound of the mother nucleus structure (Figure 1), which is on the basis of imatinib structure and to modified the structure of imatinib by computer-aided drug design [15]. CB1107...
demonstrated a good pharmacological activity. In this study, the concentration of CB1107 in mouse plasma was detected to study its absorption in vivo. The objective of the present study was to develop a high-throughput liquid chromatography-tandem mass spectrometry method to determination of CB1107 in rat plasma and to investigate its pharmacokinetics after gavage administration.

![Figure 1](image1.png)  
**Figure 1** The structure of CB1107

2 Experimental

2.1 Chemicals and reagents

CB1107 (purity > 98%, by HPLC) was prepared by Liaoning key laboratory of new drug research and development and identified by NMR techniques. HPLC grade Acetonitrile, methanol were purchased from Tedea Company Inc. (Fairfield, USA). Formic acid, ethanol were purchased from China National Medicines Corporation Ltd. (China). Ultra-pure water was purchased from Watson (Hong Kong, China).

2.2 Instrument and analytical conditions

The Liquid Chromatograph system (Agilent 1200 Series, Agilent Technologies, Palo Alto, USA) coupled to a triple-quadrupole tandem mass spectrometer (Agilent 4000 Series, Applied Biosystems, Foster City, USA). Chromatographic separation was achieved on a Zorbax Extend C18 Rapid Resolution HD column (4.6 mm × 50 mm, 1.8 µm; Agilent, Santa Clara, CA, USA). The column temperature was maintained at 35 °C and at flow rate of 0.6 mL/min. Injection volume was 20 µL. The mobile phases consisted of 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B). Gradient elution was performed using 0 min, 85.0%A; 15 min, 52.0%A; 15.5 min, 1.0%A; 18.5 min, 85%A. The gradient elution re-equilibration time was 30 min.

The HPLC-MS/MS analyses of CB1107, the ESI interface was used in positive ion mode. The optimized mass spectrometric parameters were as follows: the drying gas temperature was 325°C and these heat gas temperature was 360°C. The flow rates of drying gas and sheath gas were 9 L/min and 11 L/min, respectively. Pressure of nebulizer was set at 35 psi. Fragmentor voltage and collision energy were 300 V and 35 eV. The transitions (precursor to product) monitored were m/z 533.29 → 534.29 for CB1107 (Figure 2). The dwell time was 500 ms for each transition.

![Figure 2](image2.png)  
**Figure 2** Full-scan product ion spectra of [M+H]+ ions for CB1107

2.3 Animals

Male KM mice weighing 20±2 g, were bought from Liaoning Changsheng Biotechnology Co. LTD (Liaoning, China). Mice were given filtered tap water and commercial rat chow ad libitum. Animals fasted and drank freely for 12 hours before to the experiment. The studies
are in line with the rules of animal experimentation (State Committee of Science and Technology, China).

2.4 Preparation of standard solutions and quality control samples

Stock solutions of CB1107 was prepared in methanol (100 µg/mL) as stock solution. Next, the stock solution of CB1107 was serially diluted with methanol according to a linear concentration gradient: 0.01 µg/mL, 0.05 µg/mL, 0.1 µg/mL, 0.5 µg/mL, 1.0 µg/mL, 2.0 µg/mL and 5.0 µg/mL. All the solutions were stored at 4°C in the dark and brought to room temperature before use.

The method of calibration solution were prepared as followed: transferred 100 µL of the corresponding standard stock solution into a 5 mL Eppendorf tube, added 200 µL of plasma sample, then CB1107 calibrations standards were prepared at concentrations of 0.01 µg/mL, 0.05 µg/mL, 0.1 µg/mL, 0.5 µg/mL, 1.0 µg/mL, 2.0 µg/mL and 5.0 µg/mL with methanol. Quality control (QC) samples were prepared with blank plasma or tissue homogenates independently, and the concentrations of blank plasma and tissue homogenates were 0.01 µg/mL (low), 1 µg/mL (medium) and 5.0µg/mL (high).

2.5 Sample treatment

Serum sample was prepared using methanol as precipitant with a volumetric precipitant-to-sample ratio of 9:1, to the 200 µL serum sample, 1800 µL methanol was added and vortex-mixed 2 min, then centrifugation at 5,000 rpm for 20 min after precipitation. After centrifugation, take the supernatants filtered through 0.22 µm regenerated cellulose before 20 µL was injected into the HPLC system for analysis.

2.6 Method validation

2.6.1 Selectivity

The specificity of the method was evaluated by comparing the chromatograms of blank mouse serum, blank mice serum spiked with mixed standards, and an administrated mouse serum to ensure no endogenous interference.

2.6.2 Linearity and sensitivity

Calibration samples were prepared by 100 µl blank mouse plasma with 100 µl stock solution of CB1107 to obtain concentrations of calibration samples range from 0.01 to 5 µg/mL. The seven-point calibration curve was constructed by plotting the peak area (y) of CB1107 to concentration of CB1107 (x). The lower limit of detection (LOD) and lower limit of quantification (LOQ) were calculated using a signal-to-noise ratio of 3 and 10 times, respectively.

2.6.3 Accuracy and precision

QC samples at three of low, medium, and high concentrations were analyzed six replicates during one-day for intra-day assessed and different days for inter-day assessment. The precision was defined as the relative standard deviation (RSD) and accuracy assessed was by comparing the measured concentration to its nominal concentration.

2.6.4 Extraction recovery and matrix effects

To assess extraction recovery standards, the extracted samples (blank plasma spiked with standards and then extracted with methanol), post-extracted samples (blank serum spiked with methanol then spiked with standards ) were analyzed. The extraction recovery effect was assessed by dividing the peak areas of the extracted samples by those of the post-extracted samples. The matrix effect was assessed by comparing the peak areas of the blank serum spiked with the analytes (A) to that of pure standard solution containing equivalent amounts of the analytes (B) at three QC levels. The ratio (A/B × 100%) was used to evaluate the matrix effect.

2.6.5 Stability

The stability studies of the serum samples were tested by exposing samples to three conditions: Short-term stability was assessed at room temperature for 24 h, Freeze-thaw stability was assessed over three freeze-thaw cycles and long-term stability was assessed at storage (-80°C).
for 3 months. All analytes were considered stable when the accuracy bias was within ±15% of the nominal concentrations.

### 2.6.6 Pharmacokinetics study

For pharmacokinetic study, 90 male KM mice were randomly divided in nine groups (n = 10 per group), mice only had free access to water 12 h before administration. They were divided randomly into two days, and the mice were orally administered with CB1107 at three doses of 400, 600 and 800 mg/kg, and three mice per dose. The blood samples were collected from the orbital venous plexus at 0 h before dosing and 0.25, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 12.0 and 24.0 h after dosing. Blood samples were centrifuged at 5000 rpm for 15 min at 4°C after standing for 0.5 h, and then the upper serum fractions were obtained and immediately stored at -80°C until analysis. Plasma was harvested by centrifugation at 5000 rpm for 10 min and stored at -80°C until analysis.

### 3 Results

#### 3.1 Method validation

**3.1.1 Specificity**

The typical chromatograms of blank serum, blank serum spiked with mixed standards and serum samples from a rat at 4 h after orally administered with CB1107 are shown in Figure 3. It can be seen that the background noise was low, and no endogenous interference was observed at retention times for CB1107 (9.04 min).

![Typical chromatograms of the different samples](image)

**Figure 3** Typical chromatograms of the different samples (A) blank rat plasma, (B) plasma spiked with CB1107 at 5 µg/mL, (C) plasma 4.0 h after oral administration with 800 mg/kg.

**3.1.2 Linearity and sensitivity**

Calibration curves showed good linearity over the concentration range of 0.01-5 µg/mL for CB1107 in rat plasma. A typical linear regression equation was $y = 239169x + 2452$, $R^2 = 0.9989$, where A represents peak area and C is the plasma concentration of CB1107. The LOQ
value was 10.0 ng/mL, at which the intra- and inter-day accuracy was less than 4.10%, and the precision was < 15%. The method indicated that the assay is precise and accurate.

### 3.1.3 Accuracy and precision

QC samples at three concentration levels (0.01, 2, 5 µg/mL) are summarized in Table 1. The relative standard deviations (RSDs) of intra-day and inter-day precision were less than 4.10%. The accuracy of intra-day and inter-day were within 15%. These results indicated that this method was accurate and reproducible for the determination of CB1107 in mouse plasma.

| Sample | QC concentration (µg/mL) | Intra-day (Precision RSD, %) | Accuracy (RE, %) | Inter-day (Precision RSD, %) | Accuracy (RE, %) |
|--------|--------------------------|-----------------------------|-----------------|----------------------------|-----------------|
| Plasma | 0.01                     | 2.63                        | -8.52           | 3.03                       | -9.62           |
|        | 2.00                     | 3.93                        | -2.3            | 4.1                        | 3.21            |
|        | 5.00                     | 3.18                        | 5.63            | 3.93                       | -6.78           |

### 3.1.4 Extraction recovery and matrix effects

The extraction recoveries and matrix effects of CB1107 in serum are shown in Table 2. At three QC concentrations of CB1107, the extraction recoveries ranged from 86.5% to 96.3%, and the matrix effects ranged from 89.9% to 108.7%, and the relative standard deviations (RSDs) are in 15%.

| Sample | QC concentration (µg/mL) | Extraction recovery (n = 6) (Mean %) | RSD (%) | Matrix effects (n = 6) (Mean %) | RSD (%) |
|--------|--------------------------|--------------------------------------|---------|--------------------------------|---------|
| Plasma | 0.01                     | 96.3                                 | 3.4     | 89.9                           | 8.9     |
|        | 2.0                      | 87.3                                 | 13.0    | 108.7                          | 6.7     |
|        | 5.0                      | 86.5                                 | 11.2    | 95.0                           | 4.3     |

### 3.1.5 Stability

The stabilities of CB1107 were studied under three conditions the results are listed in Table 3. The results demonstrated that CB1107 was stable after exposure at room temperature for 24 h, after freeze-thaw cycles and storage (-80°C) for 3 months with no significant loss of activity. Importantly, CB1107 was stable during the routine analysis for the pharmacokinetics study.

| Sample | QC concentration (µg/mL) | Room temperature for 12 h (mean ± SD%) | three freeze-thaw cycles (mean ± SD%) | storage (-80°C) for 3 months (mean ± SD%) |
|--------|--------------------------|----------------------------------------|--------------------------------------|-------------------------------------------|
| Plasma | 0.01                     | 92.1± 5.2                              | 93.6±4.2                             | 93.9±2.5                                  |
|        | 2.0                      | 82.1± 3.2                              | 89.8±5.6                             | 87.6±4.3                                  |
|        | 5.0                      | 105.3±5.6                              | 105.7±3.6                            | 98.6±3.4                                  |

### 3.1.6 Pharmacokinetics

The established method of HPLC was successfully applied to pharmacokinetic studies of CB1107 after oral administration at different dose. The curves of the mean serum concentrations of CB1107 versus time after oral administration are shown in Figure 4. The pharmacokinetic characteristics were derived using DAS 2.0 software, which based on a non-compartmental model. The corresponding pharmacokinetic parameters are shown in Table 4. And the pharmacokinetic characteristics of CB1107 in mice belong to the two-compartment model. When the doses were 400 mg/kg, 600 mg/kg and 800 mg/kg, corresponding area under the plasma concentration-time curve (AUC) respectively were 20.011±1.24 mg/h/L, 26.778±2.19 mg/h/L and 38.82±1.44 mg/h/L, suggesting that CB1107 have a good absorption in the body. And the
AUC of three doses are proportional, indicating that CB1107 conforms to linear pharmacokinetics in vivo. The terminal half-life ($T_{1/2z}$) were 6.897±0.69 h, 5.617±0.42 h and 4.751±0.54 h respectively. The body clearance (CL/F) were 19.786±1.96 L/h/kg, 21.672±1.24 L/h/kg and 18.278±0.96 L/h/kg. The volume of distribution (V/F) respectively were 160.188±1.28 L/kg, 167.187±2.43 L/kg and 138.791±4.92 L/kg. And mean residence time (MRT) were 8.066±1.46 h, 7.955±1.23 h and 9.306±0.56 h.

![Figure 4](image)

**Figure 4** The mean plasma concentration–time curve of CB1107 in rats after oral administration of 400, 600 and 800 mg/kg

| Parameter | Unit | 200 mg/kg | 400 mg/kg | 800 mg/kg |
|-----------|------|-----------|-----------|-----------|
| $T_{1/2z}$ | h    | 6.897±0.69 | 5.617±0.42 | 4.751±0.54 |
| Tmax | h    | 4.000 | 4.000 | 4.000 |
| Cmax | mg/L | 2.39 | 2.59 | 3.42 |
| AUC | mg/L/h | 20.011±1.24 | 26.778±2.19 | 38.82±1.44 |
| V/F | L/kg | 160.188±1.28 | 167.187±2.43 | 138.791±4.92 |
| CL/F | L/h/kg | 115.423±8.96 | 156.377±6.84 | 183.718±12.96 |
| MRT | h | 8.066±1.46 | 7.955±1.23 | 9.306±0.56 |

4 Discussion

The ESI condition of CB1107 was optimized. Firstly, precursors and product ions of CB1107 for MRM mode analysis were selected from the characteristic mass spectra of the syringe pump. The influence of positive and negative ionization modes (ESI) on analytical sensitivity of analyte was studied. Ionization of CB1107 was more effective in positive mode than in negative mode. The mobile phase conditions were optimized, According to the peak shape, signal strength and reproducibility of the optimal selection conditions were achieved using acetonitrile and water, both containing 0.1% formic acid as solvents. The plasma samples were treated by protein precipitation, which could significantly simplify, and the treatment method meets the biological sample testing standard.

5 Conclusions

A simple, sensitive and specific HPLC–MS/MS method was established to analysis the pharmacokinetics of CB1107 in mice. And this method was successfully applied to study the pharmacokinetics at three different doses of CB1107 after oral administration in mice. In this study, the bioactivity mechanism of CB1107, by the pharmacokinetic investigation of CB1107 in rat, performed to determine the in vivo pharmacokinetic behavior to further elucidate.
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Conflict of interest

The authors declare no conflicts of interest in this work.

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