Validated UPLC-MS/MS method for the determination of ivosidenib in rat plasma: Application to a pharmacokinetic study

MINLE CHEN†, JIA XU‡, FEIFEI CHEN, QUAN ZHOU, SHUANGHU WANG and AIXIA HAN

1 Department of Pharmacy, Yueqing Hospital of Traditional Chinese Medicine, Wenzhou 325600, Zhejiang, China
2 The Laboratory of Clinical Pharmacy, The Sixth Affiliated Hospital of Wenzhou Medical University, The People’s Hospital of Lishui, Lishui 323000, Zhejiang, China

Received: April 6, 2022 • Accepted: July 9, 2022

ABSTRACT

Ivosidenib (AG-120) is an unlisted, but estimated to be valid, oral inhibitor for isocitrate dehydrogenase 1 (IDH1) in the phase I study of IDH1-mutated acute myeloid leukemia (AML) patients. This paper presents the investigation and validation of a rapid, effective, qualitative and quantitative determination method of ivosidenib in rat plasma by ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). The samples were treated using acetonitrile precipitation to remove protein influence. Then, the supernatant was extracted to analyze plasma concentration traits. In the UPLC system, acetonitrile and water containing 0.1% formic acid were selected as a cosolvent mobile phase, applying a gradient elution to isolate compounds in a C18 column. Mass detections were performed on a triple quadruple mass spectrometer in positive ion mode. Electroshock characteristic fragment ionization was used for m/z 583.95→214.53 for ivosidenib for quantitative determination, m/z 583.95→186.6 for qualitative determination, and m/z 492.06→354.55 for IS. The selectivity, linearity, stability, accuracy and precision were verified by reaching the guideline criteria from European Medicine Agency (EMA) and the Food and Drug Administration (FDA). The calibration curve was linear over the concentration range of 2–2,000 ng mL⁻¹ for ivosidenib in rat plasma with a lower limit of quantification (LLOQ) of at least 2 ng mL⁻¹. Additionally, there was no distinct matrix effect or carry-over phenomenon. The method was successfully established and applied to separate ivosidenib from plasma, with the entire analytical process being performed within 3 min for each sample, which shows high-efficiency and convenience for further studies of ivosidenib.

KEYWORDS

UPLC-MS/MS, pharmacokinetics, ivosidenib, rat

INTRODUCTION

Somatic point mutations of the metabolic enzyme isocitrate dehydrogenase 1 (IDH1) take part in many cell processes such as histone and DNA demethylation and adaption to hypoxia. IDH1 mutants have been described in an array of hematologic malignancies and solid tumors [1], endowing cancer cells new invasion ability and giving rise to the oncometabolite 2-hydroxyglutarate (2-HG) [1, 2]. The increase in the amount of 2-HG in cells that include normal cellular variation produce metabolic and epigenetic dysregulation. AG-120 (ivosidenib, (2S)-N-[(1S)-1-(2-chlorophenyl)-2-[(3,3-difluorocyclobutyl)amino]-2-oxoethyl]-1-(4-cyanopyridin-2-yl)-N-[(5-fluoropyridin-3-yl)-5-oxopyrrolidine-2-carboxamide, the first oral, small-molecule inhibitor of the mIDH1 enzyme to achieve clinical proof of concept in human trials, exhibits profound 2-HG lowering in tumor models and the ability to affect the differentiation of
primary patient acute myeloid leukemia (AML) samples. It has been approved by the Food and Drug Administration (FDA) for AML therapy [3], including relapsed or refractory AML [4].

To the best of our knowledge, there has been no research focused on ivosidenib in any animal plasma, let alone in human plasma, using any detecting technique such as the chromatography tandem mass spectrometry (LC-MS/MS) method or ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC/Q-TOFMS). Beyond this, we utilize a rapid, precise, effective method of ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) to determine ivosidenib in rat plasma. Our laboratory has mastered the method of ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) to detect sole and multiple chemical compounds [5, 6]. Our method allows the minimum detection limits of quantification to attain 2 ng mL$^{-1}$ and furnishes a better understanding of the PK/PD trait of ivosidenib in biological samples.

**EXPERIMENTAL**

**Chemicals and reagents**

A standard substance of ivosidenib and poziotinib (purity >98%) was purchased from Beijing Sunflower Pharmaceuti-
cals. HPLC-grade acetonitrile, formic acid and methanol were obtained from Merck Company (Darmstadt, Germany). All other chemicals and solvents were analytical grade. Ultra-

**Instruments and operating conditions**

Our detection method relied on an ACQUITY UPLC I-Class combined with XEVO TQD triple quadrupole mass spec-
trometer (Waters, Milford, MA, USA), equipped with an

electrospray ionization source. All system manipulations and data acquisition were performed using the Masslynx 4.1 software (Waters Corp.). Analytical separations were performed on a CORTES® UPLC C18 column (2.1 × 5.0 mm, 1.6 μm, Waters, USA), and the mobile phase consisted of 0.1% formic acid-water (solvent A) and acetonitrile (solvent B). Gradient elution was applied at a flow rate of 0.4 mL min$^{-1}$. The gradient program was applied as follows: 0–0.5 min, 10% B; 0.5–1 min, 10–30% B; 1–2 min, 30–95% B; 2–2.5 min, 95% B; 2.5–2.6 min, 95-10% B and finally recovering to primitive conditions in 0.4 min. The run time was maintained within 3 min, and only 2 μL samples were injected into the column. The autosampler room temperature was 4 °C, and the column was maintained at 40 °C.

Nitrogen was used as the desolvation gas (800 L h$^{-1}$) with the desolvation temperature of 500 °C. Argon was selected as a collision gas (50 L h$^{-1}$), and the cone voltage and collision energy were set at 35 and 20 V for ivosidenib, and 30 and 28 V for poziotinib used as internal standard (IS) with a dwell time of 0.108 s. Other parameters were set as follows: capillary voltage, 2 Kv; source temperature, 150 °C; desolvation temperature, 500 °C. Fragment ionization were performed using multiple reaction monitoring mode of m/z 583.95→214.53 for ivosidenib for quantitative determina-
tion, m/z 583.95→186.6 for qualitative determination, and m/z 492.06→354.55 for IS.

**Stock solutions, calibration standards and quality control samples**

Stock solution of ivosidenib were prepared by dissolving the standard substance in acetonitrile (10 mg mL$^{-1}$). Afterward, the stock solutions were rigidly diluted by acetonitrile to obtain calibration standards and three levels of QC samples. The stock solutions and working solutions were prepared in the same way. The series of calibration standards were set at 2, 5, 10, 20, 50, 100, 200, 500, 1,000, 2,000 ng mL$^{-1}$. The LLOQ, LQC, MQC and HQC were set at 2, 3, 300 and 1,500 ng mL$^{-1}$.

**Sample preparations**

Biopsimens should be pretreated to prevent the injected sample from introducing impurities that block the UPLC system. 90 μL plasma were spiked with 200 μL acetonitrile and 20 μL IS in 1.5 mL centrifugal tubes using a precipitation protein. After centrifuged for 10 min at a speed of 13,000 rpm, 120 μL supernatant fluid were transferred to the sample vials, and 2 μL supernatant were injected into the UPLC-MS/MS system.

**Method validations**

According to European Medicine Agency (EMA) and the FDA guidelines for bioanalytical method validation [7, 8], rigorous tests of linearity, LLOQ, selectivity, accuracy, precision, matrix effect and stability were evaluated. Each validation run, consisting of one set of calibration standards and three replicates of QC plasma samples, were performed within three consecutive days according to previous work [9–14].

Specificity was verified by comparing the chromatograms of six different blank plasma samples, the plasma spiked with ivosidenib and IS at the LLOQ level, and the rat plasma sample. UPLC-MS/MS chromatograms of the blanks and validation samples were compared for chromatographic integrity and potential interferences from endogenous substances.

The linearity of ivosidenib was counted on a blank plasma sample and a series of concentration ivosidenib standards by attenuating stock solutions. The concentration range of the calibration curve was 2–2,000 ng mL$^{-1}$ according to the internal standard method and the regression was expressed using a weighting factor of 1/x. The limits of detection and quantification were detected by the standard solution of the lowest concentration with signal-to-noise ratios of 3:1 and 10:1, respectively.

Six replicates of LLOQ (2 ng mL$^{-1}$), LQC (3 ng mL$^{-1}$), MQC (300 ng mL$^{-1}$), and HQC (1,500 ng mL$^{-1}$) were
employed to evaluate accuracy and precision. For accuracy and intra–day precision, three concentrations of QCs (LQC, MQC and HQC) were performed in the same day. Inter-day precision was tested with the same experiments over three validation days. Relative error (% RE) was used to determine accuracy, whereas relative standard deviation (% RSD) was used to express precision. The blank plasma spiked analyte was named solution A, and the pure calibration solution of analyte was named solution B. The matrix effect was evaluated by comparing the ratio of the response peaks of ivosidenib for solutions A and B. The routine was repeated three times and the matrix effect of IS was evaluated in the same way. Recovery was enumerated by comparing the chromatographic peak area of solution C (extracted QC samples from rat plasma after delivery) to solution A.

Stability assays of ivosidenib were carried out in three QC concentrations (3, 300, and 1,500 ng mL\(^{-1}\)) under different conditions with six replicates. Short-term stability was evaluated by detecting the standard solution-spiked plasma sample after extraction when stored at room temperature for 24 h and 4 \(^\circ\)C for 4 h, simultaneously. Long-term stability was assessed by detecting the standard solution-spiked plasma sample after extraction when stored at \(-20 \, ^\circ\)C for 14 d. A cycle of freezing at \(-20 \, ^\circ\)C for 2 h before thawing to liquid was performed for the freeze/thaw stability test. Following three cycles of spiked plasma samples, the freeze/thaw stability was obtained.

Carry-over was evaluated according to the results of an injection of extracted blank plasma after determining an upper limit of quantification (ULOQ). A signal should not be detected to confirm that there were no residuals in the injection pipe.

### Pharmacokinetic study

Six male Sprague-Dawley rats (220 ± 20 g) were provided by Wenzhou Medical University. Rats were bred according to the Laboratory Guidelines for Animal Care in a controlled 12 h light/dark cycle at 25 ± 3 \(^\circ\)C and a humidity of 50 ± 5\%.

The animal study was approved by the Animal Care and Use Committee of Wenzhou Medical University (No. 2017-0010). The rats were raised with food and water without limits. After 7 days of acclimatization, the rats were oral administered ivosidenib at a dose of 10 mg kg\(^{-1}\) for pharmacokinetic study. Approximately 0.5 mL blood from the tail vein was collected in heparinized tubes at 0.083, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, and 36 h. The blood tubes were centrifuged at 4,000 rpm at 4 \(^\circ\)C for 10 min to separate plasma, and the collected plasma was stored at \(-80 \, ^\circ\)C before analysis. The concentration-time curve and pharmacokinetic parameters were calculated using DAS software (Drug and Statistics software, version 3.2.8, The People’s Hospital of Lishui, China).

### RESULTS AND DISCUSSION

#### Optimization of UPLC-MS/MS conditions

To gain the optimized spectrogram of ivosidenib and IS, the choices of mobile phase and column were optimized by multiple injections until obtaining the optimized peak. A nonpolar CORTECS\(^{\circledR}\) BEH C18 column was selected as the stationary phase due to its high intercolumn reproducibility and separation efficiency. Methanol, formic acid, and acetonitrile were alternative options to explore as optimum mobile phases. As a result, a cosolvent of acetonitrile-water (0.1\% formic acid) was used as a mobile phase to acquire a preferable peak shape and sensitivity. The mean retention times of ivosidenib and IS were 1.94 and 1.56 min, respectively. The course of the mass spectrum to obtain precursor-to-product ion pairs was relatively sophisticated. Fragment ionization were performed using multiple reaction monitoring mode of m/z 583.95→214.53 for ivosidenib for quantitative determination, m/z 583.95→186.6 for qualitative determination, and m/z 492.06→354.55 for IS, as shown in Fig. 1.

There were some significant steps in the analytical procedure, and extracting the substance of interest from biological specimens is an issue of crucial importance for qualitative and quantitative analysis [15]. Most of the
methods had the problems associated with large volumes or
time consumption. Even a liquid-liquid microextraction
required 600 μL of organic solvent, and only 100 μL extract
were obtained as a result [16]. In our project, an organic solvent
protein precipitation extractive agent was selected to facilitate a
shorter extraction time and low amount of organic solvent.

Specificity

The representative chromatograms of blank plasma, a plasma
sample spiked with ivosidenib and IS, and a plasma sample
obtained at 4 h after oral administration of 10 mg kg\(^{-1}\)
ivosidenib were shown in Fig. 2. No interfering peaks were
observed in the chromatograms of plasma samples at the
retention times of ivosidenib or IS.

Linearity and LLOQ

The calibration curve was composed of 10-point concentra-
tions of ivosidenib standard solution ranging from 2 to
2,000 ng mL\(^{-1}\) dispersed in blank plasma, for which the
linear regression equation was 0.0006594x+0.0002941 (\(r^2 =
0.999\)). The LLOQ of ivosidenib was determined to be
2 ng mL\(^{-1}\). The RSD of precision and accuracy at LLOQ
was less than 20%, which indicated that the method was
sufficiently sensitive for our analyses.

Accuracy and precision

Precision and accuracy were determined by verifying three
concentration levels on three different days. Six sets of four
levels of QC in plasma were disposed for analysis. The values
were expressed as percent relative standard deviation (RSD,
%). It was required that the mean values of both accuracy and
precision should be within 15% of the nominal value. The
results range from −3.2 to 13.13% and are listed in Table 1,
which indicated that the intra- and inter-day accuracy and
precision of ivosidenib met our acceptance criteria.

Recovery and matrix effect

The mean peak areas of the ivosidenib spiked before extrac-
tion divided by the areas of the ivosidenib spiked after
extraction were calculated as the recovery, which evaluated
whether there was an endogenous substance interference with
the aim ionization signal response. The method was feasible
in that the three level QCs, LOQ (3 ng mL\(^{-1}\)), MOQ
(300 ng mL\(^{-1}\)) and HOQ (1,500 ng mL\(^{-1}\)) were all within
the bioanalytical method validation. Plasma-added ivosidenib
samples were compared to the ivosidenib standard solution in
the absence of a matrix to measure the matrix effect in the
three levels QCs for the six groups. By calculating the ratio of
the peak area in the presence of matrix to a pure solution of
ivosidenib, all numerical values were not greater than 15%,
certifying that the matrix did not impact the detection results.

Stability

Stability was ensured for every step in the storage and
treatment procedures by comparing concentrations of the
stock solution and working solution at each point to the
concentration of the newly developed solution of the same
labeled concentration. Through the experiments, ivosidenib

Fig. 2. Representative UPLC-MS/MS chromatograms of ivosidenib
and poziotinib (IS), (a) blank plasma; (b) blank plasma spiked with
ivosidenib (2 ng mL\(^{-1}\)) and IS (50 ng mL\(^{-1}\)); (c) rat plasma samples
taken orally 4 h after a dose of 10 mg kg\(^{-1}\) ivosidenib
stock and working solutions were confirmed to be stable in the short-term (<24 h at room temperature and <4 h at 4 °C) and long-term (<14 d at −20°C). Simultaneously, the response of plasma spiked with three levels of QC samples that experienced three freeze-thaw cycles were similar to that of the standards. The data for the stability of ivosidenib were listed in Table 2. Consequently, ivosidenib was stable in most storage and handling circumstances.

Carry over
The peak area of the blank sample following the highest point concentration of the calibration curve injections was <20% of LLOQ. Study samples could be randomized; however, a test that is in order was our habitual practice.

Application to pharmacokinetic study
The UPLC-MS/MS method for ivosidenib was validated to measure concentrations in plasma samples. Orally administered dosages of 10 mg kg⁻¹ were delivered to SD male rats (n = 6). The mean plasma concentration-time curve of ivosidenib after oral administration (10 mg kg⁻¹) in rats is shown in Fig. 3. The concentration-time curve was collected and offered propositional pharmacokinetic information. Parameters including the area under the curve (AUC), maximum concentration (Cmax), time to maximum concentration (tmax), half-life of elimination (t1/2), and clearance rate (Cl) were listed in Table 3.

Table 1. Precision, accuracy, and recovery for ivosidenib of QC samples in rat plasma (n = 6)

<table>
<thead>
<tr>
<th>Concentration (ng mL⁻¹)</th>
<th>RSD (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intra-day</td>
<td>Inter-day</td>
</tr>
<tr>
<td>Add</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Measured</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>11.39</td>
<td>11.87</td>
</tr>
<tr>
<td>3</td>
<td>5.77</td>
<td>8.67</td>
</tr>
<tr>
<td>300</td>
<td>4.08</td>
<td>12.53</td>
</tr>
<tr>
<td>1,500</td>
<td>3.19</td>
<td>13.13</td>
</tr>
</tbody>
</table>

Table 2. Summary of stability of ivosidenib under various storage conditions (n = 6)

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Concentration (ng mL⁻¹)</th>
<th>RSD (%)</th>
<th>RE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Add</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Measured</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Freeze/Thaw</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.11 ± 0.37</td>
<td>11.80</td>
<td>3.75</td>
</tr>
<tr>
<td>300</td>
<td>306.29 ± 14.00</td>
<td>4.57</td>
<td>2.10</td>
</tr>
<tr>
<td>1,500</td>
<td>1,531.72 ± 30.74</td>
<td>2.01</td>
<td>2.11</td>
</tr>
<tr>
<td>Room Temperature, 24 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.19 ± 0.17</td>
<td>5.45</td>
<td>6.41</td>
</tr>
<tr>
<td>300</td>
<td>307.00 ± 15.20</td>
<td>4.95</td>
<td>2.33</td>
</tr>
<tr>
<td>1,500</td>
<td>1,470.34 ± 61.16</td>
<td>4.16</td>
<td>−1.98</td>
</tr>
<tr>
<td>4 °C, 4 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.90 ± 0.24</td>
<td>8.22</td>
<td>−3.28</td>
</tr>
<tr>
<td>300</td>
<td>315.20 ± 7.52</td>
<td>2.39</td>
<td>5.07</td>
</tr>
<tr>
<td>1,500</td>
<td>1,529.79 ± 34.09</td>
<td>2.23</td>
<td>1.99</td>
</tr>
<tr>
<td>−20 °C, 14 d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.94 ± 0.31</td>
<td>10.69</td>
<td>−2.14</td>
</tr>
<tr>
<td>300</td>
<td>312.98 ± 12.66</td>
<td>4.05</td>
<td>4.33</td>
</tr>
<tr>
<td>1,500</td>
<td>1,538.60 ± 27.53</td>
<td>1.79</td>
<td>2.57</td>
</tr>
</tbody>
</table>

Table 3. Primary pharmacokinetic parameters after oral administration of ivosidenib in rats (n = 6)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Oral administration (n = 6)</th>
<th>RSD/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC(0-t)</td>
<td>µg/L·h</td>
<td>21,469.34 ± 2,412.73</td>
<td>11.2</td>
</tr>
<tr>
<td>AUC(0-∞)</td>
<td>µg/L·h</td>
<td>21,589.837 ± 2,415.51</td>
<td>11.2</td>
</tr>
<tr>
<td>MRT (0-t)</td>
<td>h</td>
<td>9.128 ± 0.4</td>
<td>4.3</td>
</tr>
<tr>
<td>MRT (0-∞)</td>
<td>h</td>
<td>9.316 ± 0.55</td>
<td>5.9</td>
</tr>
<tr>
<td>t1/2</td>
<td>h</td>
<td>4.149 ± 0.92</td>
<td>22.2</td>
</tr>
<tr>
<td>Tmax</td>
<td>h</td>
<td>6 ± 0</td>
<td>0</td>
</tr>
<tr>
<td>Vz/F</td>
<td>L kg⁻¹</td>
<td>2.797 ± 0.64</td>
<td>22.9</td>
</tr>
<tr>
<td>CLz/F</td>
<td>L h⁻¹ kg⁻¹</td>
<td>0.468 ± 0.05</td>
<td>10.5</td>
</tr>
<tr>
<td>Cmax</td>
<td>µg/L</td>
<td>1,879.1 ± 340.72</td>
<td>18.1</td>
</tr>
</tbody>
</table>

Fig. 3. Mean plasma concentration-time curve of ivosidenib after oral administration (10 mg kg⁻¹) in rats (n = 6, mean ± SD)

CONCLUSION
A reliable and high-throughput UPLC-MS/MS method was developed for the determination of ivosidenib, a neoteric IDH1 enzyme inhibitor, in rat plasma and its pharmacokinetic study. Protein precipitation for plasma sample preparation reveals the accurate quantitation of ivosidenib with a perfect peak with only 2 µL of injection volume. Good specificity, concordant accuracy and precision demonstrated the method feasibility. The correlation information from rat plasma could provide assistance in clinical use.

Conflict of interest: The authors declare no conflict of interest.

Ethical statement: All applicable institutional guidelines for the care and use of animals were followed. This study was...
approved by Laboratory Animal Ethics Committee Committee of Wenzhou Medical University & Laboratory Animal Centre of Wenzhou Medical University (wydw 2019-0651).

ACKNOWLEDGMENTS

This work was supported by grants funded by Public Welfare Technology Research Funding Project of Zhejiang (LGF20H150008, LGD20H060001 and LGF21H310001), City level key research and development Project of Lishui (2020ZDYF12, 2021ZDYF13 and 2021ZDYF15) and Medical and Health Research Project of Zhejiang province (2022RC303 and 2022RC302).

REFERENCES