Gradient Elution LC–MS Determination of Dasatinib in Rat Plasma and Its Pharmacokinetic Study

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Summary. A sensitive and simple liquid chromatography–electrospray ionization–mass spectrometry (LC–ESI–MS) method for determination of dasatinib in rat plasma using one-step protein precipitation was developed. After addition of carbamazepine as internal standard (IS), protein precipitation by acetonitrile was used as sample preparation. Chromatographic separation was achieved on an SB-C18 (2.1 mm × 150 mm, 5 μm) column with methanol–0.1% formic acid as mobile phase with gradient elution. Electrospray ionization (ESI) source was applied and operated in positive ion mode; selective ion monitoring (SIM) mode was used to quantification using target fragment ions m/z 488.2 for dasatinib and m/z 338.7 for the IS. Calibration plots were linear over the range of 10–1000 ng mL−1 for dasatinib in rat plasma. Lower limit of quantification (LLOQ) for dasatinib was 10 ng mL−1. Mean recovery of dasatinib from plasma was in the range 82.2%–93.6%. Relative standard deviation (RSD) of intra-day and inter-day precision were both less than 8%. This developed method is successfully used in pharmacokinetic study of dasatinib in rats.

Key Words: LC–ESI–MS, rat plasma, gradient elution, dasatinib

Introduction

Dasatinib (BMS-354825), N-(2-chloro-6-methylphenyl)-2-[[6-[4-(hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide monohydrate (Fig. 1), is effective therapy for chronic myeloid leukemia (CML) after imatinib failure. It is a novel, oral, multitargeted kinase inhibitor that is active against breakpoint cluster region–abelson (BCR-ABL) and Src family kinases (SFKs). Unlike imatinib and its derivative, nilotinib (AMN107), dasatinib binds to multiple conformations (active and inactive) of the ABL kinase, is active against 21 of 22 tested BCR-ABL [1–4] mutations conferring imatinib resistance, and potently inhibits the SFKs implicated in imatinib resistance. However, it produces some side effects, such as diarrhea, peripheral edema, fatigue, nausea, headache, and pyrexia. The most common adverse events (AEs) related to treatment were diarrhea and gas-
trointestinal bleeding. Therefore, it is important to develop an analytical method for the determination of dasatinib for therapeutic drug monitoring.

In recent years, numerous laboratories have reported the use of high-throughput bioanalytical procedures for the quantification of antileukemia drugs [5, 6], a small number of analytical methods have been reported for dasatinib based on high-performance thin-layer chromatography (HPTLC) or high-performance liquid chromatography (HPLC) or radioactive labeling methods [7–9] and a liquid chromatography–mass spectrometry (LC–MS) method for plasma determination of this agent [10–13]. As with most approaches used to quantify pharmaceuticals, analytical methods for measuring tyrosine kinase inhibitor levels have focused on each agent in isolation and generally have been applied to pharmaceutical analysis or plasma determination of the agent.

Fig. 1. Chemical structures of dasatinib (a) and carbamazepine (IS, b)
High-performance liquid chromatography–ultraviolet (HPLC–UV) method has performed limited sensitivity and specificity and required a relatively long analysis time to attain sufficient chromatographic separation. In recent years, the high sensitivity and selectivity of tandem mass spectrometry (MS/MS) had led to a growing trend of developing fast analytical methods. The scope of our work was to develop and optimize a simple, easy to use, sensitive, and specific LC–MS method for the determination of dasatinib in rat plasma. The developed method was successfully applied to pharmacokinetic studies of dasatinib in rats following intragastric administration.

Experimental

Chemicals and Reagents

Dasatinib (purity >98.0%) was a gift from Shanghai Institute of Pharmaceutical Industry (Shanghai, China), and carbamazepine (purity >98.0%) was purchased from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). LC-grade acetonitrile and methanol were from Merck Company (Darmstadt, Germany). While LC-grade formic acid was from Tedia Company (Cincinnati, USA), ultra-pure water was prepared by a Millipore Milli-Q purification system (Bedford, MA, USA).

Instrumentation and Conditions

All analyses were performed with a 1200 Series liquid chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a quaternary pump, a degasser, an autosampler, a thermostated column compartment, and a Bruker Esquire HCT ion-trap mass spectrometer (Bruker Technologies, Bremen, Germany) equipped with an electrospray ion source and controlled by ChemStation software (Version B.01.03 [204], Agilent Technologies, Waldbronn, Germany).

Chromatographic separation was achieved on an Agilent Zorbax SB-C18 (2.1 mm × 150 mm, 5 μm) column at 40 °C, with methanol–0.1% formic acid as mobile phase. The flow rate was 0.4 mL min⁻¹. The HPLC gradient profile can be seen in Table I.
Table I. HPLC gradient for detection of dasatinib in rat plasma

<table>
<thead>
<tr>
<th>Load time (min)</th>
<th>Pump flow (μL min⁻¹)</th>
<th>Formic acid %</th>
<th>Acetonitrile %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>400</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>1.0</td>
<td>400</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>4.0</td>
<td>400</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>7.0</td>
<td>400</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>8.0</td>
<td>400</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>13.0</td>
<td>400</td>
<td>90</td>
<td>10</td>
</tr>
</tbody>
</table>

Drying gas flow and nebulizer pressure were set at 6 L min⁻¹ and 25 psi. Dry gas temperature and capillary voltage of the system were adjusted at
350 °C and 3500 V, respectively. LC–MS performed with SIM mode was used to quantification using target fragment ions \( m/z \) 488.2 for dasatinib and \( m/z \) 338.7 for carbamazepine (IS), in positive ion electrospray ionization interface (Fig. 2).

**Calibration Standards and Quality Control Samples**

Individual stock solutions of dasatinib (1.0 mg mL\(^{-1}\)) and carbamazepine (IS) (100 µg mL\(^{-1}\)) were prepared in methanol. Working solutions for calibration and controls were prepared from the stock solution by dilution using methanol. About 2.0 µg mL\(^{-1}\) working standard solution of IS was prepared by dilution of the IS stock solution with methanol. All of the solutions were stored at 4°C and were brought to room temperature before use.

Dasatinib calibration standards were prepared by spiking blank rat plasma with appropriate amounts of the working solutions. Calibration plots were constructed in the range 10–2000 ng mL\(^{-1}\) for dasatinib in rat plasma (concentrations 10, 20, 50, 100, 200, 500 and 1000 ng mL\(^{-1}\)). Quality-control (QC) samples were prepared by the same way as the calibration standards, three different plasma concentrations (20, 200, and 1000 ng mL\(^{-1}\)). The analytical standards and QC samples were stored at −20°C.

**Sample Preparation**

Before analysis, the plasma sample was thawed to room temperature. In a 1.5-mL centrifuge tube, an aliquot of 10 µL of the IS working solution (2.0 µg mL\(^{-1}\)) was added to 100 µL of collected plasma sample followed by the addition of 200 µL acetonitrile. The tubes were vortex mixed for 0.5 min. After centrifugation at 14,900 g for 10 min, the supernatant (5 µL) was injected into the LC–MS system for analysis.

**Method Validation**

The selectivity of the method was evaluated by analyzing blank rat plasma, blank plasma spiked with dasatinib and IS, and a rat plasma sample.

Calibration curves were constructed by analyzing spiked calibration samples on three separate days. Peak area ratios of dasatinib to IS were plotted against analyte concentrations, and standard curves were well fitted to the equations by linear regression with a weighting factor of the reciprocal of the concentration (1/\(x\)) in the concentration range of 10–1000 ng mL\(^{-1}\).
To evaluate the matrix effect (ME), blank rat plasma was protein precipitated and then spiked with the analyte at 20, 200, and 1000 ng mL$^{-1}$. The corresponding peak areas were then compared to those of neat standard solutions at equivalent concentrations, and this peak area ratio is defined as the matrix effect. The matrix effect of IS was evaluated at the working concentration (200 ng mL$^{-1}$) in the same manner.

Accuracy and precision were assessed by the determination of QC samples at three concentration levels in six replicates (20, 200, and 1000 ng mL$^{-1}$) in three validation days. The precision was expressed by relative standard deviation (RSD) and the accuracy by relative error (RE).

The recoveries of dasatinib at three QC levels ($n = 6$) were determined by comparing peak-area of the analytes in QC samples to which the analytes were added post-protein precipitation at equivalent concentrations. The recovery of the IS was determined in a similar way.

The stabilities of dasatinib in rat plasma were evaluated by analyzing three replicates of plasma samples at the concentrations of 20, 200, and 1000 ng mL$^{-1}$, which were exposed to different conditions. The short-term stability was determined after the exposure of the spiked samples at room temperature for 2 h and the ready-to-inject samples (after protein precipitation) in the HPLC autosampler at room temperature for 24 h. The freeze–thaw stability was evaluated after three complete freeze–thaw cycles (−20 to 25 °C) on consecutive days. The long-term stability was assessed after storage of the standard spiked plasma samples at −20 °C for 30 days.

**Pharmacokinetic Study**

Male Sprague–Dawley rats (200–220 g) were obtained from Laboratory Animal Center of Wenzhou Medical College (Wenzhou, China) and were used to study the pharmacokinetics of dasatinib. All six rats were housed at Wenzhou Medical College Laboratory Animal Research Center. All experimental procedures and protocols were reviewed and approved by the Animal Care and Use Committee of Wenzhou Medical College and were in accordance with the Guide for the Care and Use of Laboratory Animals. Animals were housed under controlled conditions (25 ± 1 °C, RH 55 ± 10%) with a natural light–dark cycle. They were allowed to adapt to the housing environment for at least 1 week before the study. Diet was prohibited for 12 h before the experiment, but water was freely available. Blood samples (0.3 mL) were collected from the tail vein into heparinized 1.5 mL polythene tubes at 0, 0.16667, 0.33333, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 h after oral administration of dasatinib (15 mg kg$^{-1}$). The samples were immediately centrifuged at 2500 g for 5 min. The plasma obtained (100 μL) was
stored at −20 °C until analysis. Plasma dasatinib concentration versus time data for each rat was analyzed by DAS software (Version 2.0, Wenzhou Medical College, China).

Results and Discussion

Selectivity and Matrix Effect

Fig. 3 shows the typical chromatograms of a blank plasma sample and a blank plasma sample spiked with dasatinib and IS. No interfering endogenous substances were observed at the retention times of the analyte and IS.

Fig. 3. LC-ESI-MS chromatograms of dasatinib (1) and carbamazepine (IS, 2), (a) blank plasma; (b) blank plasma spiked with dasatinib (200 ng mL⁻¹) and IS (200 ng mL⁻¹)
The ME for dasatinib at concentrations of 20, 200, and 1000 ng mL\(^{-1}\) were measured to be 105.5 ± 4.8, 100.7 ± 6.2 and 105.1 ± 3.8% \((n = 6)\), respectively. The ME for IS (200 ng mL\(^{-1}\)) was 108.6 ± 6.6% \((n = 6)\). As a result, ME from plasma was negligible in this method.

**Calibration Curve and Sensitivity**

The linear regressions of the peak area ratios versus concentrations were fitted over the concentration range 10–1000 ng mL\(^{-1}\) for dasatinib in rat plasma. Typical equation of the calibration curve was: \(y = 0.002057x + 0.002444, r = 0.999136 \,(n = 3)\), where \(y\) represents the ratios of dasatinib peak area to that of IS and \(x\) represents the plasma concentration. For dasatinib, the present LC–MS method gave an LLOQ of 10 ng mL\(^{-1}\) with an accuracy of 8.8% in terms of RE and a precision of 9.2% in terms of RSD. It was more sensitive than that in literatures [9, 11]; LOQ was 100 ng mL\(^{-1}\) for dasatinib in plasma reported by Pirro E. [9], and LOQ was 62.5 ng mL\(^{-1}\) for dasatinib by De Francia S. [11].

**Precision, Accuracy, and Recovery**

The precision of the method was determined by calculating RSD for QCs at three concentration levels over three validation days. Intra-day precision was 4% or less, and the inter-day precision was 8% or less at each QC level (20, 200, and 1000 ng mL\(^{-1}\)). The accuracy of the method ranged from 98.6% to 107.5% at each QC level.

Assay performance data are presented in Table II. The above results demonstrate that the values are within the acceptable range, and the method is accurate and precise.

*Table II. Precision, accuracy and recovery for dasatinib of quality control sample in rat plasma \((n = 6)\)*

<table>
<thead>
<tr>
<th>Concentration (ng mL(^{-1}))</th>
<th>RSD (%)</th>
<th>RE (%)</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intra-day</td>
<td>Inter-day</td>
<td>Intra-day</td>
</tr>
<tr>
<td>20</td>
<td>3.6</td>
<td>6.2</td>
<td>4.2</td>
</tr>
<tr>
<td>200</td>
<td>2.9</td>
<td>1.9</td>
<td>2.1</td>
</tr>
<tr>
<td>1000</td>
<td>2.9</td>
<td>7.9</td>
<td>5.0</td>
</tr>
</tbody>
</table>
Mean recoveries of dasatinib were better than 82.2%, and the recovery of the IS was 94.5 ± 4.9% (n = 6).

**Stability**

The auto-sampler, room temperature, freeze–thaw, and long-term (30 days) stability results indicated that the analyte was stable under the storage conditions described above since the bias in concentration was within ±15% of their nominal values, and the established method was suitable for the pharmacokinetic study.

**Application of the Method**

The method was applied to a pharmacokinetic study in rats. The main pharmacokinetic parameters from the noncompartmental model analysis were summarized in Table III. The mean plasma concentration–time curve after oral administration of a single 15 mg kg⁻¹ of dasatinib was shown in Fig. 4.

![Fig. 4](image-url)  
*Fig. 4. Mean (±SD) plasma concentration time profile with noncompartmental model after oral administration of dasatinib (15 mg kg⁻¹) in six rats.*
Table III. The main pharmacokinetic parameters after oral administration of single dosage 15 mg kg\(^{-1}\) dasatinib to rats (\(n = 6\))

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Mean (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(t_{1/2}) (h)</td>
<td>10.2 ± 3.7</td>
</tr>
<tr>
<td>Cl (L h(^{-1}))</td>
<td>9.6 ± 3.0</td>
</tr>
<tr>
<td>V (L kg(^{-1}))</td>
<td>129.3 ± 22.5</td>
</tr>
<tr>
<td>(C_{\text{max}}) (ng mL(^{-1}))</td>
<td>238.9 ± 55.9</td>
</tr>
<tr>
<td>AUC(_{0-t}) (h ng mL(^{-1}))</td>
<td>1300.2 ± 203.4</td>
</tr>
<tr>
<td>AUC(_{0-\infty}) (h ng mL(^{-1}))</td>
<td>1665.7 ± 445.4</td>
</tr>
</tbody>
</table>

Conclusion

A sensitive, simple, and specific LC–MS method with gradient elution for the determination of dasatinib in rat plasma was developed and validated over the concentration range of 10–1000 ng mL\(^{-1}\). The method was validated to meet the requirements for pharmacokinetic determination of the dasatinib in rat plasma.

References


Accepted by DA