A Validated UPLC-MS/MS Method for Therapeutic Drug Monitoring of Sorafenib in Patients with Hepatocellular Carcinoma

Chia-Ni Lin1,2, Chun-Nan Yeh3, Yun-Fen Huang1, Ya-Ching Huang1,2 Hsiao-Chen Ning1,2

1 Department of Laboratory Medicine, Chang Gung Memorial Hospital, Taoyuan, Taiwan
2 Department of Medical Biotechnology and Laboratory Science, College of Medicine, Chang Gung University, Taoyuan, Taiwan
3 Department of Surgery, Chang Gung Memorial Hospital and University, Taoyuan, Taiwan

Introduction

Hepatocellular carcinoma (HCC) represents a global health problem and the incidence of this cancer in patient with cirrhosis is still increasing in several countries. It is the sixth most prevalent cancer and the third most common cause of cancer mortality worldwide. There was no effective treatment available for patients diagnosed at advanced stage or who progressed into an advanced stage after other treatments failed. Sorafenib is the first small-molecule drug approved by FDA for treatment of the advanced HCC. Sorafenib is an oral multikinase inhibitor with activity against Raf-1, B-Raf, VEGFR2, PDGFR and c-Kit receptors, has a potent antiangiogenic and proapoptotic activity and therefore presents a marked antitumor effect. A given dose of sorafenib can result in different plasma concentrations which may lead to sub-therapeutic drug exposure or increase adverse drug reactions at excessive plasma concentrations. Skin toxicities, including hand-foot skin reaction (HFSR), are primary side effects of new multikinase inhibitors. Although not life threatening, HFSR can severely impact the physical, psychological and social well-being of patients receiving multiple tyrosine kinase inhibitors and can lead to dose reduction and discontinuation, which may potentially negate the life-prolong effects of multiple kinase inhibitors. The mechanism of adverse event, including HFSR, is still unclear and needs to be fully clarified. To analyze advanced HCC patients treated with sorafenib and validate its efficacy and safety, a method to quantitate sorafenib is essential. The purpose of this study is to establish and validate a UPLC-MS/MS based assay to quantitate the sorafenib concentration in blood.

Materials and Methods

Chemicals and Reagents
- Sorafenib Tosylate was obtained from Bayer AG (Leverkusen, Germany)
- Sorafenib-d3 was from Toronto Research Chemicals. (Toronto ON, Canada)

UPLC-MS/MS
- Serum was precipitated with acetonitrile containing sorafenib-d3
- Supernatant was injected into the UPLC-MS/MS
- MP-A: 5 mM ammonium formate (pH 3.5) ; MP-B: Acetonitrile
- 0.5 mL/min flow rate, 4.5 min gradient LC program,
- Column: 50 x 2.1 mm, 1.7 μm BEH C18 ;Column oven 40℃
- Instrument :

Waters Acquity Ultra Performance Liquid Chromatograph coupled with a Waters Xevo TQ-S triple-quadrupole mass spectrometry (Milford MA, USA)

Results

Ion suppression
Ion suppression was evaluated by analyzing extracted samples while infusing. No significant suppression was noted.

Linearity
The linearity was estimated using 11 equally spaced samples with duplicates according to the procedure described in CLSI document EP06AE. The linearity of the analytical range obtained with patient serum was linear in the range of 19.5 – 10279.5 ng/mL with a correlation coefficient of 0.9995.

LOQ
Samples were prepared with serum spiked with sorafenib. The limit of detection was the lowest concentration for which all qualitative parameters meet acceptable criteria: acceptable chromatography, retention time within ± 2 %, all monitored ions present and all ion mass ratios within ± 20 %. The LOQ for this assay was 19.5 ng/mL.

Carryover
Carryover was evaluated by analyzing a set of high concentration at 9389 ng/mL and low concentration at 347 ng/mL in serum in the order as per the CLSI protocol EP10-A2. There was no carryover observed in this assay.

Figure 1. Extraction ion chromatograms for calibrator at concentration 1000 ng/mL

Accuracy
To evaluate accuracy, drug-free human serum was pooled and used as a matrix to spike samples with 300, 1200 and 3000 ng/mL of sorafenib. The recovery was 100.8%, 102.6% and 103.9 % respectively.

Imprecision
Imprecision was determined by analyzing three levels of control material prepared in duplicate for 14 days.

<table>
<thead>
<tr>
<th>Sorafenib (ng/mL)</th>
<th>Within-Run</th>
<th>Between-Run</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>C I</td>
<td>352.6</td>
<td>4.6</td>
</tr>
<tr>
<td>C II</td>
<td>1360.2</td>
<td>16.5</td>
</tr>
<tr>
<td>C III</td>
<td>3397.6</td>
<td>39.6</td>
</tr>
</tbody>
</table>

In conclusion, a fast and accurate UPLC-MS/MS method was developed and can be applied for routine therapeutic drug monitoring purposes in patients treated with sorafenib.