A sensitive and specific liquid chromatographic-tandem mass spectrometric (LC/MS/MS) method capable of quantifying KX2-391 in human plasma is described.

In this method, the drug was extracted from a 0.1 mL sample of plasma using a liquid-liquid extraction method. Chromatography separation was performed on a reverse phase C18 column. Detection was achieved using an AB SCIEX API-4000 tandem mass spectrometer employing turbo-ion spray ionization in the positive ion mode along with multiple reaction monitoring (MRM). The lower limit of quantitation was 0.1 ng/mL. The method has been successfully applied to clinical pharmacokinetic studies.

Introduction

KX2-391 (KX01) is a highly selective Src kinase inhibitor that has demonstrated efficacy in pre-clinical animal models of colon, pancreatic, prostate and breast cancer. This is the first substrate-targeted kinase inhibitor to enter clinical trials and is expected to have improved efficacy with reduced toxicity. It belongs to an emerging new family of targeted cancer agents.

Liquid Chromatography:

HPLC System: Shimadzu LC-10AD
Analytical Column: C18 column, 2.00 x 50 mm, 5 µm
Mobile Phase A: Water with 10 mM ammonium formate, pH 4
Mobile Phase B: Acetonitrile
Flow rate: 0.6 mL/min
Injection Volume: 20 µL

Mass Spectrometry

MS System: AB Sciex API-4000
Condition: LC+(-)ESI-MS/MS (MRM)
MRM Transition:
KX2-391: 432.5 → 113.5
KX2-377: 449.9 → 113.5

Results and Discussion

Table I. Validation Data Summary

<table>
<thead>
<tr>
<th>Condition</th>
<th>RE%</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeze/Thaw</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Cycles, -70°C</td>
<td>&lt;10.7</td>
<td></td>
</tr>
<tr>
<td>60 Days, -70°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 Days, -70°C</td>
<td>&lt;10.7</td>
<td></td>
</tr>
<tr>
<td>Atmosphere</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pressure, 35 psi</td>
<td>&lt;10.7</td>
<td></td>
</tr>
</tbody>
</table>

A simple, sensitive and selective LC-MS/MS method was developed and validated for quantifying KX2-391 in human plasma. A lower limit of quantitation of 0.1 ng/mL was established utilizing a plasma sample volume of 0.1 mL. This method has been successfully applied to the analysis of clinical samples.