

Overview

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

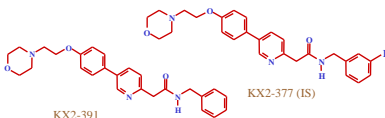
In this method, the drug was extracted from a 0.1 mL sample of plasma using a liquid-liquid extract method. Chromatography separation was performed on a reverse phase C18 column. Detection was achieved using an AB SCIEX API-4000 tandem mass spectrometer employing turbo-ion spray ionization in the positive 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 is belong to an emerging new family of targeted cancer treatments. Currently, there is no published bioanalytical method for the determination of KX2-391 in human plasma. A short sample preparation time and a sub ng/mL detection limit were required to support ongoing clinical trials. We now report a rapid, specific, and highly sensitive liquid chromatographic-tandem mass spectrometric (LC-MS/MS) method capable of quantifying KX2-391 from 0.1 mL of human plasma at levels as low as 0.1 ng/mL.

Structures



Experimental

Sample Preparation

Each plasma sample aliquot (0.1 mL) was transferred into a 96 deep-well plate. The internal standard was added to the sample well as a *tert*-butyl methyl ether (MTBE, 0.7 mL) solution, followed by ammonium hydroxide (0.040 mL of 10% solution). Both additions were conducted by using a Tomtec® liquid handling system. The 96 deep-well plate was covered with a sealing mat. A plate shaker was used to vigorously vortex the sample plate.

The samples were centrifuged at approximately 4,000 rpm for 10 min. The supernatant (0.6 mL) was transferred into a second 96 deep-well plate using a Tomtec® system. The transfer was followed by solvent evaporation using SPE-Dry 96® with temperature set at about 35 °C and nitrogen stream pressure at about 35 psi. The plate residues were reconstituted with 0.250 mL of reconstitute solution (1% acetic acid in acetonitrile:water in 1:1 ratio). The 96 deep-well plate was covered with a sealing mat. A plate shaker was used to vigorously vortex the sample plate.

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
 Gradient
 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 → 113.8

Results and Discussion

Table I. Validation Data Summary

Calibration Range		0.1 to 50 ng/mL	
Correlation coefficient (r, 3 batches)		0.9938 to 0.9945	
Accuracy & Precision		Accuracy	Precision
QC	Conc. (ng/mL)	RE%	CV%
LLOQ	0.1	7.00	7.08
Low	0.3	4.33	10.8
Medium	20	0.50	9.20
High	40	1.25	7.57
Method Recovery		Compared with Nominal Value (%)	
		≥72.00	
Freeze/Thaw		Condition	Accuracy RE%
Bench-To		3 Cycles, -70 °C	<10.0
Autosampler Extract Stability		4 hrs, Room Temperature	<1.75
Long-Term Storage Stability		2 Days, Room Temperature	<2.25
		100 Days, -70 °C	<10.7

- Atmosphere pressure chemical ionization (APCI) and electrospray ionization (ESI) modes were tested for their response; Positive ESI was found to provide better sensitivity. The precursor ion mass spectrum and product ion mass spectrum are shown in Figure 3. The analyte and internal standard MRM transitions are listed under the LC/MS conditions. The instrument was tuned to give maximum abundance of each compound's product ion. Figure 1 presents two traces: the first shows a typical blank human plasma chromatogram, and the second shows a human plasma sample with KX2-391 (0.1 ng/mL).

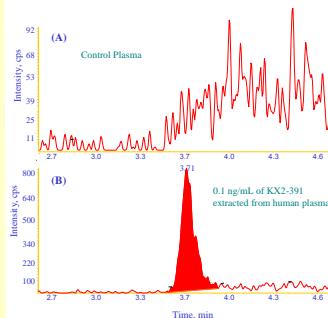


Figure 1. Ion chromatograms of blank plasma sample (A) and 0.1 ng/mL KX2-391 extracted from plasma (B)

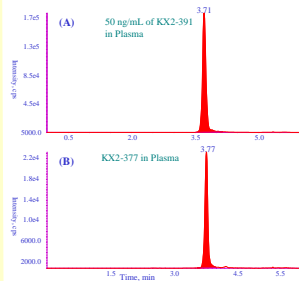


Figure 2. Ion chromatograms of 50 ng/mL KX2-391 extracted from plasma (A), and the internal standard extracted from plasma (B)

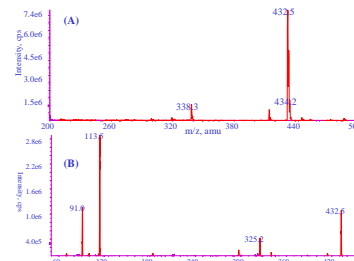


Figure 3. LC(+)-ESI-MS spectrum (A) and product ion scan (B) of KX2-391

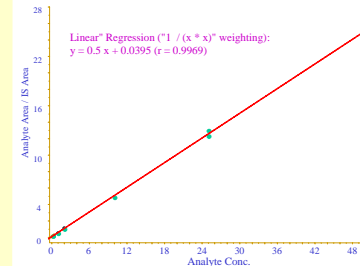


Figure 4. Representative calibration curve for the determination of KX2-391 in the range from 0.1 ng/mL to 50 ng/mL in human plasma

- A simple, robust liquid-liquid extraction method was used to prepare the samples for analysis. Sample preparation could be done quickly and consistently through the use of 96-well plates with the Tomtec® liquid handling system.
- Excellent linearity was obtained with a correlation coefficient equal to or greater than 0.9938. The inter-day precision (CV%) and accuracy (RE%) were ≤7.00% and ≤10.8% respectively, for all QC samples, including the lower limit of quantitation (LLOQ) (Table I). KX2-391 was found to be stable during the assessment of QC samples over the following conditions: (1) Three freeze/thaw cycles and (2) storage at -70 °C for about 3 months. Extracted samples were found to be stable at room temperature for ~2 d.

Conclusions

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.