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Determination of Crizotinib in Human and Mouse Plasma by Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC-ESI-MS/MS)

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Abstract

An LC-ESI-MS/MS method using high-throughput solid-phase extraction (SPE) was developed and validated to measure crizotinib in human and mouse plasma to support ongoing clinical and preclinical pharmacokinetic studies. Chromatographic separation of mouse or human plasma extracts was performed on a Supelco Discovery c18 column (50 × 2.1mm, 5.0 μ) with gradient elution using a combination of acidified aqueous and methanol (MeOH) mobile phases. The mass-to-charge transition monitored for detection and quantitation of crizotinib was m/z 450.2>260.2 while the stable label internal standard (ISTD) was monitored at m/z 457.2>267.3. The validation studies demonstrated that the assay is both precise and accurate with %CV < 9% and accuracies within 8% of nominal target concentration across all concentrations tested for both the human and mouse plasma matrices. Sample volumes required for analysis were 50 μL and 25 μL for human plasma and mouse plasma, respectively. Calibration curves were linear over a range of 5 – 5000 ng/mL for human plasma and 2 – 2000 ng/mL for mouse plasma. The use of a 96-well plate format enabled rapid extraction as well as compatibility with automated workflows. The method was successfully applied to analyze crizotinib concentrations in plasma samples collected from children enrolled on a phase I pediatric study investigating the use of crizotinib for treatment of pediatric brain tumors.

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Keywords

Crizotinib; human plasma; Solid Phase Extraction (SPE); liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS); lower limit of quantitation (LLOQ); pharmacokinetic studies

1. Introduction

The human hepatocyte growth factor (HGF) and its receptor c-Met play essential roles in normal physiological development (embryogenesis and organogenesis) and wound repair [1]. However, abnormal dysregulation of HGF and c-Met signaling has also emerged as an important characteristic in multiple cancers [2–6]. In adult high grade glioma, extensive preclinical and clinical data have been published about the crucial role of HGF/c-Met in the tumorigenesis, tumor growth, angiogenesis, and cell migration.[7]. In pediatric glioma research, a recent genome-wide analysis of 43 tumor samples from 40 children with diffuse intrinsic pontine glioma (DIPG) revealed *MET* was the second most common amplified oncogene in DIPG (11/43; 26%) [7].

Crizotinib, an orally bioavailable small molecule inhibitor of c-Met and anaplastic lymphoma kinase (ALK), has been approved by the FDA for the treatment of ALK-positive non-small cell lung cancer (NSCLC) [8–10]. Because of data implicating the c-Met pathway activation in adult high-grade gliomas and in children with diffuse intrinsic pontine glioma [7,11–13], crizotinib is currently under evaluation in a phase I pediatric study (SJHG12; ClinicalTrials.gov number NCT01644773) in combination with dasatinib for treatment of diffuse intrinsic pontine glioma (DIPG) or high-grade glioma (HGG).

The pharmacokinetic disposition of crizotinib is unknown in pediatric patients with malignant brain tumors. The novel combination of dasatinib and crizotinib poses a potential for pharmacokinetic interactions because crizotinib is a moderate inhibitor of CYP3A, and hepatic metabolism of both agents is largely dependent on CYP3A (unpublished data). Hence, an accurate and precise bioanalytical assay will be essential for analyzing pharmacokinetic study samples in this patient cohort. In turn, these pharmacokinetic data will be used for refining dosing in future clinical trials of this combination regimen in children.

Several publications provide brief descriptions of crizotinib bioanalytical assays, but do not provide full methodological details, including validation data [14,15]. A recent report described the first validated assay for crizotinib in mouse plasma using protein precipitation and LC–MS/MS [16], however, no validated methods have been published for use with human samples. Therefore, in this paper we describe a rapid LC-MS/MS method that was developed and validated according to internal SOP's to assay crizotinib concentrations in both human and mouse plasma using a 96-well solid phase extraction procedure. Concentration-time data derived using this method will be critical for defining the pharmacokinetic disposition of crizotinib in combination with dasatinib and interpreting toxicity and disease response data from the ongoing pediatric phase I trial.

2. Experimental

2.1. Chemicals

Crizotinib (99.5% purity) and ISTD ($[^2\text{H}_5, ^{13}\text{C}_2]$ -Crizotinib 99% purity) were obtained from Alsachim (Illkirch Graffenstade, France). Methanol was obtained from Fisher Scientific (Fairlawn, NJ, USA) and Formic acid (FA, 98% purity) was purchased from Fluka BioChemika (Buchs, Switzerland). Blank human plasma was obtained from Life Blood (Memphis, TN). All water was purified using a Millipore Milli-Q UV plus and Ultra-Pure Water System (Tokyo, Japan). Other chemicals were purchased from standard sources and were of the highest quality available.

2.2. Apparatus and conditions

2.2.1 Chromatographic conditions—The HPLC system consisted of a Shimadzu (Kyoto, Japan) system controller (CBM-20A), pump (LC-20ADXR), autoinjector (SIL-20AC), online degasser (DGU-20A3), and column heater (CTO-20AC).

Chromatographic separation was performed at 50 °C using a Discovery c18 column (50 × 2.1mm, 5.0 μ; Supelco, USA). The analyte and ISTD were eluted using a gradient with mobile phase A consisting of (water/formic acid 100:0.3 v/v) and mobile phase B (MeOH/formic acid 100/0.3 v/v). The gradient starting conditions were 20% mobile phase B and 80% mobile phase A. The starting conditions were held for 0.5 minutes then the conditions were changed to 30% mobile phase B from 0.5 to 1 minute and held until 4 minutes when the %B was increased to 85%. At 4.5 minutes the system was returned to starting conditions for a total sample run time of 7.0 minutes.

To demonstrate the method's ability to tolerate small changes likely to be encountered during routine use, two additional mobile phases were prepared to assess the impact of mobile phase acid alterations on crizotinib quantitation. A curve and QC's (low, medium, and high, n=3) were extracted and injected with three different mobile phase preparations. One mobile phase was consistent with the description above, and the two other mobile phases were used with solvent to acid ratios of 100:0.2 v/v and 100:0.4 v/v.

2.2.2. Mass spectrometric conditions—Mass spectra were obtained using an AB SCIEX API 5500Qtrap (Toronto, Canada) with an ESI source. The software used to operate the mass spectrometer was Analyst (Version 1.5.1, Applied Biosystems, Foster City, CA). The instrument was operated using multiple reaction monitoring (MRM) and positive ion mode with unit resolution for both Q1 and Q3. The optimized MS/MS conditions were: ion spray source temperature at 650 °C, curtain (CUR) gas pressure at 25 psi, both gas 1 (GS1) and gas 2 (GS2) pressure at 70.0 psi, ionspray voltage (IS) at 5500 V, collision-activated dissociation (CAD) set at medium, declustering potential (DP) at 88 V, entrance potential (EP) at 11.0 V, collision energy (CE) at 35.0 V, and collision exit potential (CXP) at 16 V. The transitions monitored for quantitation were m/z 450.2>260.2 for crizotinib and m/z 457.2>267.3 for the ISTD. Transition m/z 450.2>177.1 was monitored qualitatively as confirmatory fragment using the same instrument parameters with the exception of CE which was set at 50.0 V.

2.3. Patient Sample Collection and Handling for Pharmacokinetic Analysis

Serial blood samples for crizotinib pharmacokinetic analysis were obtained from a pediatric patient on course 1 day 1 of therapy before the oral dose and at 1, 2 (± 0.5), 4 (± 1), 8 (± 2), 24 (± 4), and 48 (± 4) hours after the administration of crizotinib. At each specified time point, three milliliters of blood were drawn, placed in a K₂-EDTA vacutainer and mixed thoroughly. Samples were centrifuged at room temperature (10,000 $\times$ g for 2 minutes) within 30 minutes of collection, and the plasma supernatant transferred to labeled tubes and stored at -80°C until further analysis.

2.4. Sample preparation

2.4.1. Stock solutions—Stock solutions were prepared by dissolving crizotinib and ISTD in MeOH:H₂O 80/20 v/v to concentrations of 1 mg/mL. The crizotinib stock solution was diluted to prepare 2 intermediates for both human plasma (10,000 ng/mL and 100,000 ng/mL) and mouse plasma (5,000 ng/mL and 50,000 ng/mL) in MeOH:H₂O 50/50 (v/v). A separate working solution was made for each calibrator and quality control concentration by diluting the intermediates to the applicable concentration with MeOH:H₂O 50/50 v/v. The ISTD working solution was prepared in the same manner to a concentration of 1000 ng/mL. All solutions were stored at 4°C .

2.4.2. Calibration curve and quality controls—To prepare calibration samples, 50 μL of blank matrix was spiked with an appropriate amount of stock solution to obtain concentrations of 5, 10, 100, 500, 1000, 2000, 4000, and 5000 ng/mL. In mouse plasma, the samples were prepared in the same fashion but at 2, 4, 40, 200, 400, 800, 1600, and 2000 ng/mL. Both calibration curves were designed to roughly reflect the expected range of sample concentrations in the clinical and preclinical pharmacokinetic studies. Three quality control concentrations were prepared at 15, 750, and 3500 ng/mL for human plasma and 6, 300, and 1400 ng/mL for mouse plasma in the same manner as the calibration samples.

2.4.3. Sample preparation—Solid-phase extraction (SPE) was performed with an Oasis HLB Microelution 96-well plate (Waters, USA). 50 μL aliquots of human plasma were spiked with 10 μL of ISTD working solution. To all samples calibrators and controls, 125 μL of 4% phosphoric acid was added prior to extraction to reduce protein-drug interactions. To verify the robustness of this approach, QC's were also extracted using 3% and 5% phosphoric acid as well as 4% formic acid.

All SPE wells were conditioned with 200 μL of MeOH followed by 200 μL of H₂O. The samples diluted with the phosphoric acid solution were then loaded and washed twice with 200 μL of H₂O. Samples were eluted with 100 μL of MeOH, and after collection, dried down completely in approximately 15 minutes under house nitrogen gas. Sample residues were then reconstituted with 300 μL of a solution that mirrored the starting conditions of the gradient (MeOH:H₂O 20/80 v/v) and up to 5 μL injected on the LC-MS/MS system for analysis. Human and mouse plasma samples were processed in the same manner with the exception that we used 25 μL aliquots of plasma, 100 μL of 4% phosphoric acid solution, and 100 μL of reconstitution solution for murine samples. The reduced sample volume was due to limitations in the volume that can be obtained from the mice.

2.5. Method validation

2.5.1 Linearity and lower limit of quantitation—Linearity was evaluated with calibration curves constructed of 8 unique concentrations. Least square linear regression weighted with $1/x^2$ and the coefficient of determination (r^2) was used to evaluate the linearity of each calibration curve. The LLOQ of the method was defined as the lowest concentration in the calibration curve that had accuracy within 20% of the theoretical value and a signal/noise (S/N) ratio of at least 5.

2.5.2 Accuracy, precision, and recovery—The accuracy and precision were evaluated with intra-day studies as well as inter-day studies that spanned three days. The following four concentrations were studied for inter- and intra-day accuracy/precision: LLOQ (5, 2 ng/mL), low (15, 6 ng/mL), medium (750, 300 ng/mL), and high (3500, 1400 ng/mL) concentration (n=6 at each level) for human and mouse plasma along with a freshly prepared curve each day. A dilution study was also included in the human plasma validation by diluting a sample 10x the high QC (35000 ng/mL) in sextuplicate. The acceptance criterion used as pass/fail for the studies was $\pm 20\%$ of nominal concentration at the LLOQ and $\pm 15\%$ for all other levels with %CV of $< 20\%$ at the LLOQ and $< 15\%$ for all other levels. Recovery was assessed by calculating the ratio of the instrument response of extracted low (15, 6 ng/mL) and high (3500, 1400 ng/mL) concentration samples to that of samples prepared by spiking blank extracted matrix at the same concentrations.

2.5.3 Selectivity, carryover, and matrix effects—Selectivity in both human and mouse plasma was assessed by extraction of LLOQ and blank samples derived from six different plasma lots. Since dasatanib was administered as a concomitant medication beginning on day 15 of our clinical protocol, calibration curves and controls (n=3) at low, medium, and high concentrations were extracted using pooled human plasma spiked with a dasatanib concentration reflective of the C_{max} (100 ng/mL; unpublished data) to rule out potential interference with crizotinib quantification. In addition, 3 blank samples of the pooled plasma containing dasatanib were extracted to identify possible interfering peaks. Sample carryover was evaluated by observing chromatograms of blank extracted samples injected immediately following the high standard (5000, 2000 ng/mL). Matrix effects were evaluated by comparing post extraction spiked samples to mobile phase spiked samples. The matrix factor (MF) was calculated by dividing the instrument response of the post extracted samples by the instrument response of the mobile phase spiked samples. The matrix effects were measured at low and high concentrations and were evaluated in 6 different lots of pooled human plasma. This approach for assessing matrix effects was selected based on previous published studies in this area [17]. Matrix effects in mouse plasma were evaluated in the same manner as the human plasma.

2.5.4 Stability—Stability studies were conducted under conditions appropriate for routine sample handling. Pools of spiked human plasma and mouse samples, prepared at low and high concentrations (15, 3500 ng/mL for human and 6, 1400 ng/mL for mouse), were aliquotted. Three samples of each concentration were analyzed immediately following preparation and the average concentration was used to establish a time 0 concentration for reference. All remaining aliquots were stored separately at either 4 °C, ambient temperature,

or -80°C . These samples were extracted and analyzed at the appropriate time points and compared against the time 0 concentrations. To assess the autosampler stability window, reconstituted sample extracts were stored at both room temperature and 4°C . They were then assayed at the designated time point.

3. Results and discussion

3.1. Chromatography

The first step in developing the chromatography of the method was selecting the optimal analytical column. Based on work with other agents of similar physicochemical properties we elected to use a c18 column for chromatographic separation. After testing several different columns with our basic scouting gradients, the Discovery c18 ($50 \times 2.1\text{mm}$, $5.0\ \mu$) column provided the best combination of peak shape and run time. In particular, we observed significantly reduced tailing with this column in comparison to other columns. In preliminary scouting gradients, both MeOH and acetonitrile (ACN) were tested as mobile phase with MeOH providing significantly better chromatography. Addition of 0.3% formic acid and maintaining the column at 50°C served to improve consistency of peak shape and reduce retention time. A coflumn flush was added after analyte elution as a precaution to prevent late eluting peak carryover due to the low organic content (30%) used to elute the analyte. Examples of typical chromatograms are shown in Figure 1.

3.2. Mass spectrometry

Sample analysis was implemented on an AB Sciex API-5500QTrap LC/MS/MS system at unit (Q1) and unit (Q3) resolution. The instrument was set for positive ionization mode and multiple reaction monitoring (MRM). All mass spectrometry parameters were optimized via infusion of reference standard. Q1 scans were used to select the parent ion and product ion scans were used to select the transition ions. The source of the fragments were confirmed with precursor scans shown in Figures 2 and 3. The scans shown demonstrate that the source of the fragments monitored were from a parent mass consistent with crizotinib and the ISTD. This is also consistent with previous literature report [16].

3.3 Sample Preparation

SPE using an Oasis HLB Microelution 96-well plate was selected for sample extraction. The oasis HLB Microelution plate was selected for its ability to handle small sample volumes. This has the added benefit of reducing the volumes of solvent required saving money and minimizing waste. Utilizing the 96-well format also makes it amenable to implementation in automated workflows. Solvent choice for sample preparation was based on manufacturer recommendations.

3.4. Precision, accuracy and recovery

The intra-day and inter-day precision and accuracy results are presented in Table 1. Precision is represented as %CV and the accuracy as %error (i.e. % deviation from nominal). These results indicate the method is both accurate and precise with intra-day precision $<4\%$ and the accuracy within 6% of nominal concentrations for all levels in human plasma. For the inter-day studies in human plasma, precision was $<6\%$ and the accuracy

within 4% for all levels. The dilution QC's were accurate within 12% and a precision of <3%. The recovery of the analyte and ISTD agreed well and was >82% in human plasma. In mouse plasma, precision for all studies was <9% and accuracy was within 8%. Recovery was >60% for both the analyte and ISTD. The high %CV for the high QC's could be attributed to one sample that was likely due to a poor injection. Even with the higher variance of the recovery in the high QC's, the behavior of crizotinib and the ISTD closely mirrored one another. The recovery results are reported in Table 2.

3.5. Linearity and lower limit of quantitation

The crizotinib calibration curves in human plasma were linear from 5 to 5000 ng/mL with correlation coefficients (r^2) greater than 0.998. For human plasma, all LLOQ samples measured from both intra-day and inter-day varied by less than 13% from the expected concentration with an overall average accuracy of 94.4% and 96.0%, respectively. At the LLOQ, S/N 85 was typical. The crizotinib calibration curves in mouse plasma were also linear with r^2 values greater than 0.999 while all LLOQ samples deviated by less than 14% with an average of 102.0% and 104.4% for intra-day and inter-day respectively. S/N at the LLOQ in mouse plasma was typically 18.

3.6. Selectivity, carryover, and matrix effects

Selectivity of the assay was confirmed by monitoring both extracted blank human plasma and LLOQ samples for any coeluting peaks in six different lots of plasma. In both human and mouse plasma matrices, we observed no coeluting peaks for either crizotinib or the ISTD. In addition, crizotinib free human plasma samples containing 100 ng/mL of dasatinib were analyzed along with a set of crizotinib curve and QC's that were also prepared using pooled plasma containing 100 ng/mL of dasatinib. This dasatinib concentration was chosen based upon the observed C_{max} for dasatinib in patients on this study (unpublished observations). Comparing these samples to a similar set prepared in blank plasma without dasatinib, there were no interfering peaks observed in the blank extracted samples and the quantitation of crizotinib in QC samples was similar. A summary of the concentration of the QC's prepared using the dasatinib spiked plasma calculated on both sets of curves is reported in Table 3. The % change between the calculated concentrations using the 2 curves was less than 4% for all three levels of QC's.

Minimal matrix effects were detected for both human (MF=0.937) and mouse (MF=1.07) plasma. The MF for the stable label ISTD agreed very well with the analyte and had an identical MF as crizotinib in human plasma and was nearly identical at 1.05 in mouse plasma. The results from the matrix effects studies are listed in Table 2. Carryover was assessed by monitoring blank samples after injection of the high standard. Carryover was observed in the human plasma assay development and ranged from 10–25% of the LLOQ. The underlying issue was traced back to the autosampler contamination and the problem appeared to be concentration dependent. To account for the carryover, runs where carryover exceeded 20% of the LLOQ during system suitability testing were run with blank injections between samples. Carryover was not an issue with the mouse plasma assay, likely due to the lower concentrations of calibrator samples.

3.7. Stability

Samples were defined to be stable if they deviated by less than 15% from the time 0 samples. Results of the stability studies indicate crizotinib to be stable in both mouse and human plasma matrices when stored at 4 °C. Stability was established at ambient room temperature for up to 24 hours for human plasma and 5.5 hours for mouse plasma. Crizotinib human plasma samples were also stable through three freeze-thaw cycles. Stability in mouse plasma was only established for one freeze-thaw cycle since we typically do not have sufficient sample volume for repeat analysis. Extract stability when reconstituted in mobile phase was confirmed to be at least 5 days.

3.8. Application of method to clinical pharmacokinetic study

To demonstrate the applicability of the method described in this report, we analyzed plasma samples from a child enrolled on the phase I pharmacokinetic study of crizotinib, SJHG12 (ClinicalTrials.gov number NCT01644773). After administration of an oral crizotinib dosage of 130 mg/m² on course 1 day 1 of therapy, serial blood samples were collected and processed at predefined timepoints as described in *Patient Sample Collection and Handling for Pharmacokinetic Analysis*. After bioanalysis, a two-compartment model was fit to the crizotinib plasma concentration-time data using maximum likelihood estimation as implemented in ADAPT. Pharmacokinetic parameters estimated included elimination rate, absorption rate constant, and intercompartmental rate terms. A representative plasma concentration-time curve for this patient is shown in Figure 4, including the observed data and the model fit demonstrating the applicability of the crizotinib LC MS/MS method.

3.9. Comparison to existing methods

Contrasting the previously published mouse plasma method and the method described in this report, the key differences include sample preparation, chromatography platforms, and the linear curve range. While the prior published method uses protein precipitation, the present method employs a SPE sample preparation using a 96-well format. This approach is advantageous compared to protein precipitation methods in terms of cleaner sample extracts as well as compatibility with most automated sample extraction systems for high-throughput sample analysis. Despite that the previously published assay utilizes UPLC separation (instead of HPLC), we were still able to develop an assay with a retention time under 2 minutes. Both assays have a 1000 fold curve range, however our method has a lower LLOQ at 2 ng/mL, as opposed to 10 ng/mL [16].

3.10. Robustness

Three separate mobile phases with varying concentrations of formic acid were prepared to assess the impact of acid concentration on crizotinib quantitation. The slopes of the curves from the three sets of injections were 0.00618, 0.00616, and 0.0059 respectively. The %CV of the three curves was 2.6% and all QC levels in all three injections met acceptance criteria as described in section 2.5.2. A second experiment tested the ability of the assay to tolerate variations in the acid used during sample preparation. QC's (Low and High, n=3) were extracted using 3%, 4%, and 5% phosphoric acid as well as 4% formic acid. Extracted along with the QC's was a fresh curve used for quantitation. All sets of QC's met pre-

defined acceptance criteria. The %CV for the sets of low and high QC's was 3.2% and 4.4% respectively. The low variability between the QC's shows that the method can withstand variability in acid concentration and still produce accurate and consistent results. These two experiments provide support for the assay's ability to tolerate small changes and still perform properly during routine assay applications.

4. Conclusion

In this report, we describe for the first time, a validated assay for quantitation of crizotinib in human plasma samples using LC-ESI-MS/MS. The method proved to be both accurate and precise for human as well as murine plasma samples with key advantages of being fast, simple, reproducible, and compatible with automation. The LLOQ of 5 ng/mL for human plasma and 2 ng/mL for mouse plasma provided an excellent signal to noise ratio, providing the potential to measure lower concentrations, if required. In this report, we have also successfully applied this bioanalytical method by measuring crizotinib in human plasma collected from a child enrolled on a phase I study evaluating crizotinib for patients diagnosed with malignant brain tumors. Data derived from this assay will be critical for defining future crizotinib dosing regimens in children.

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Highlights

- A novel method for the determination of crizotinib by lc/ms/ms is described.
- The method was validated in human plasma and mouse plasma.
- The curve ranges were 5–5000 ng/ml and 2–2000 ng/ml for human and mouse plasma respectively.
- The method was applied to a clinical pharmacokinetic study of children treated with crizotinib.

Figure 1.

Chromatograms of A.) crizotinib scan in blank human plasma, B.) ISTD scan in blank human plasma, C.) LLOQ in mouse plasma RT 1.86, D.) ISTD in mouse plasma RT 1.86, E.) LLOQ in human plasma RT 1.95, F.) ISTD in human plasma RT 1.95.

Figure 2.
Precursor ion scan of 260.2 m/z for crizotinib.

Figure 3.

Precursor ion scan of 267.3 m/z for [2H₅,¹³C₂]-Crizotinib.

Figure 4.
Crizotinib plasma concentration–time profile in a pediatric patient after one oral dose of crizotinib (130 mg/m²).

Table 1
Accuracy and Precision Results

Inter-day and intra-day accuracy and precision results for quantitation of crizotinib in human and mouse plasma (n=6).

Concentration (ng/ml)	Inter-day		Intra-day	
	CV (%)	Accuracy*	CV (%)	Accuracy (%)
Human Plasma				
5	4.37	96.0 % (4.80 ng/mL)	1.81	94.4 % (4.72 ng/mL)
15	3.64	99.0 % (14.9 ng/mL)	2.68	97.8 % (14.7 ng/mL)
750	5.58	98.9 % (742 ng/mL)	3.28	98.3 % (737 ng/mL)
3500	3.48	99.9 % (3500 ng/mL)	1.86	98.2 % (3440 ng/mL)
35000	N/A	N/A	2.25	111.5 % (39000 ng/mL)
Mouse Plasma				
2	4.59	104.8 % (2.10 ng/mL)	1.96	102.0 % (2.04 ng/mL)
6	5.91	97.3 % (5.84 ng/mL)	8.32	92.7 % (5.56 ng/mL)
300	4.19	100.2 % (301 ng/mL)	4.08	101.1 % (303 ng/mL)
1400	3.38	100.1 % (1400 ng/mL)	3.10	102.1 % (1430 ng/mL)

* Concentrations reported to 3 significant figures

Table 2

Recovery and Matrix Factor

Matrix effects and recovery in human and mouse plasma.

	Human Plasma				Mouse Plasma			
	Recovery		MF		Recovery		MF	
	CV (%)	Avg. (%)	CV (%)	Avg. (%)	CV (%)	Avg. (%)	CV (%)	Avg. (%)
Crizotinib								
Low QC*	4.47	84.3	9.39	0.954	12.0	79.7	3.39	1.12
High QC†	3.64	90.5	9.70	0.920	71.0	64.2	1.61	1.01
Combined	4.06	87.4	9.55	0.937	41.5	72.0	2.50	1.07
ISTD								
Low QC*	7.05	82.1	10.2	0.979	4.71	81.0	3.24	1.12
High QC†	7.07	91.4	11.9	0.894	71.9	62.7	3.36	0.976
Combined	7.06	86.8	11.1	0.937	38.3	71.9	3.30	1.05

* Crizotinib conc. 15 ng/mL for human 6 ng/mL for mouse ; ISTD 200 ng/mL

† Crizotinib conc. 3500 ng/mL for human and 1400 ng/mL for mouse ; ISTD 200 ng/mL

Table 3
Calculated Concentration of QC's Prepared in Plasma Spiked With 100 ng/mL of Dasatanib

Results for comparison of calibrator's and QC's prepared in matrix with and without dasatanib.

Nominal Concentration (ng/mL)	Concentration Using Curve 1 [*] (ng/mL)	Concentration Using Curve 2 (ng/mL)	% Change
(n=3)			
15	16.8	16.4	-2.4
750	815.0	784.7	-3.7
3500	3876.7	3730.0	-3.8

* Curve 1 contained 100 ng/mL of dasatanib in the plasma.