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Quantification of asciminib by LC-MS/MS method in human plasma: Validation and stability studies

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ABSTRACT
An accurate, sensitive, and linear liquid chromatographic tandem mass spectrometric method was developed for the quantification of asciminib in human plasma. Voriconazole, internal standard (IS), and asciminib were isolated from the plasma samples by C_{18}-cartridges. Chromatographic isolation was processed with Atlantis/dC_{18} (2.1 × 100 mm, 3 µm) stationary phase and a mobile solvent system of formic acid (0.1%) and methyl alcohol (20:80, v:v), conveyed at a 0.7 ml/minute flowing rate. Analytes were quantified by ionization in a positive approach with electrospray ionization at the mass transitions of (m/z): asciminib, 450.11/239.09, and voriconazole (IS), 350.3/281.1. No interference by constituents of plasma blank or other components was detected. The association between asciminib concentration levels and their respective peak response fractions to voriconazole was rectilinear between 39.0 and 1,586.0 ng/ml. Intraday and interday precisions were ≤4.79% for asciminib. The interday bias was between −4.28% and 5.76%, and the intraday bias was −3.75% and 4.53%. The mean measured extraction recovery of asciminib was 99.06%. The recovery of IS was 98.34%. Asciminib was subjected to long-term, bench-top, freeze-thaw, short-term stability, dry extract, auto-sampling, and stock solution stability at Low quality control and High quality control levels, and it was stable at all these conditions. The established technique can be utilized for the regular quantification of asciminib in plasma samples in industries, forensic laboratories, and clinical research organizations.

1. INTRODUCTION

Asciminib is a tyrosine kinase inhibitor that is utilized in the treatment of chronic-phase Ph+ chronic myeloid leukemia (CML). Specifically, it inhibits the ABL1 kinase functioning of BCR-ABL 1 fused protein, which drives CML amplification in the majority of diseased patients. It has demonstrated efficacy in Ph+ CML with a mutation of T315I, which generates resistance to treatment mutant BCR-ABL 1 compared to wild-type BCR-ABL 1. The progression of CML is predominantly driven by the Philadelphia chromosome translocation, which generates a fusion oncogenic gene [1], BCR ABL 1, between the ABL and BCR genes [2]. The combination of this gene results in the production of a fusion protein, BCR-ABL1, with transforming activities and increased tyrosine kinases that contribute to the proliferation of CML [3].

Asciminib allosteric inhibitor of the BCR ABL 1 tyrosine kinase. Because it attaches to the myristoyl pockets of an ABL 1 part of the fusion proteins, it may block the oncogenic activity that would otherwise be caused by the fusion proteins by trapping it into an inactive conformation [4]. The steady-state AUC_{tau} and C_{max} at a dose of 40 mg twice daily were 5,262 ng.hour/ml and 793 ng/ml, respectively. It is chemically named as *N*-4-[chloro (difluoro) methoxy] phenyl]-6-[(3*R*)-3-hydroxy pyrrolidin-1-yl]-5-(1*H*-pyrazo l-5-yl) pyridine-3-carboxamide; hydrochloride, with chemical formula (Figure 1) and weight [5,6] of $C_{20}H_{18}ClF_2N_5O_3$ and 449.84 g·mol^{-1}.

Literature on asciminib reveals that no analytical approaches were reported for the quantification of asciminib by liquid chromatographic tandem mass spectrometric (LC-MS/MS). Therefore, there was a need for an analytical method for the quantification of asciminib in the biosamples with a high degree of sensitivity. The current study directed the development

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of an LC-MS/MS method for the determination of asciminib in samples of plasma.

2. MATERIAL AND METHODS

2.1. Reagent chemicals

Asciminib was gifted by Novartis India Ltd., Mumbai. India. HCOOH of analytical quality and methyl alcohol and can (acetonitrile) of LC grade were acquired from JT Bakers in Hyderabad, India. The Milli-Q®RO system's built-in water supply was used for the preparation of the moveable and washing solvent systems.

2.2. LC-MS/MS instrument

Analytes have been extracted out of plasma samples with standard C-18 Sep-Pak tubes made by Waters Corporation, MA, USA. Chromatographic elution was done on an LC-MS/MS instrument with a Waters 2695 Alliances separating model (Waters Corporation, USA) for sample introduction and delivery of solvent, Micro mass Quattromicro API triple quadruple mass spectrometric system connected to an Electro spray ionization (ESI) (Z-spray) source as detection system (Micromass, Manchester, UK). At room temperature, an analysis was done with RPAtlantis dC18 (2.1 × 100 mm, 3 µm) column that was covered by a guard column (3.9 × 20 mm, 5 µm). Mass Lynx software (Version 4.0) running on Microsoft Windows XP professionals was employed to handle the device, get the data, measure the signals-to-noise fraction, and integrate and smoothen peaks.

2.3. LC and mass system conditions

Chromatographic isolation was processed with Atlantis/dC_{18} (2.1 × 100 mm, 3 µm) stationary phase and mobile solvent system of formic acid (0.1%) and methyl alcohol (20:80, v:v), conveyed at 0.7 ml/minute flow rate. The ESI interface has functioned in ionization of positive mode. The following mass constraints were utilized: 400°C: desolvation temperature, 150°C: source temperature, and 3.1 kV: capillary voltage. Cone and desolvation gas of N_2 was employed at a flow of 50 and 800 l $hour^{-1}$, correspondingly. Collisional gas of argon was processed at 0.17 ml/minute flow in a collision cell. Collisional energy and voltages of the cone for asciminib and voriconazole were optimized as 20/34 and 16/24 V, respectively. Analytes were quantified by ionization in a positive approach with electro-spray ionization at the mass transitions of (m/z): asciminib, 450.11/239.09, and voriconazole [internal standard (IS)], 350.3/281.1.

2.4. Quality control (QC) and standard sample solutions

Asciminib and IS stock solutions (1,000 µg/ml) were executed in the mobile phase separately. They were dissolved even more with plasma to make workable solutions with a concentration of 10 g/ml. Human plasma was used to make eight calibration standards with concentration levels from 39 to 1,586 ng/ml and four QC sample solutions with concentrations of 39, 111, 793, and 1,189 ng/ml [7]. QCs and calibration standards were stirred for 1 minute, and then 1 ml aliquots were poured into borosilicate glass (13 × 100 mm) culture tubing and preserved at −20˚C till they were needed.

2.5. Sample processing

Allow 1 ml samples of QC, calibration curve, and blank plasma samples to attain room temperatures. The end concentration of each tube was 85 ng/ml, so 250 µl of the IS solution was incorporated and each of the tubes was shaken for 20 seconds. Before samples were put on the C18 Sep-Pak cartridges, they were treated with one milliliter of methyl alcohol and then 2 ml water. Then 1 ml was combined with water (1 ml) and sample solutions were isolated with 1 ml of 0.1% HCOOH in methyl alcohol [8]. The samples were then dried out under light streams of N_2 gas in a block of heat at 45°C. The leftovers were diluted with100 µl of movable solvent system, placed in an auto-sampling container, and 10 µl was loaded into LC-MS/MS equipment.

2.6. Stability study

By keeping the processed QC samples in an auto sampler that was kept at a temperature of 5°C ± 3°C for a time of 2 days, 20 hours, and 27 minutes, it was possible to assess their auto sampler stability. By screening at −20°C for 2 months, long-term stabilities for the analyte and IS were processed at Low quality control (LQC) and High quality control (HQC). By holding at 2°C–8°C for 10 days, stock solution stability for the analyte was processed at LQC and HQC levels. Three cycles of freeze and thaw stability processing were performed at −20°C and room temperature [9]. The drug and IS were treated for stability of short-term by placing them at room conditions for 8 hours. A 17 hours, 28 minutes storage duration at room temperature was used to test the benchtop condition of the QC spiked sample solutions. The QC spiked samples for dry extract stability were assessed for the duration of 2 days by placing those samples at −28 C ± 5 C.

2.7. Validation of the analytical method

The technique was validated in accordance with the accepted practices outlined in the US FDA guidelines [10] on the validation of bioanalytical methods. Specificity, accuracy, linearity, precision, stability, and recovery were among the validation criteria [11,12].

3. RESULT AND DISCUSSION

3.1. Mass and LC conditions optimization

The precursor and product ions of asciminib and voriconazole were examined by introducing the standard methanolic solution (1.0 µg/ml) into a mass system utilizing a syringe pump with 20 µl flow. Analytes were quantified by ionization in a positive approach with electro-spray ionization in Multiple reaction monitoring (MRM) mode at the mass transitions of (m/z): asciminib, 450.11/239.09 and voriconazole (IS), 350.3/281.1. The following mass constraints were utilized: 400°C: desolvation temperature, 150°C: source temperature, and 3.1 kV: capillary voltage. Cone and desolvation gas of N_2 was employed at a flow of 50 and 800 l $hour^{-1}$, correspondingly. Collisional gas of argon was processed at 0.17 ml/minute flow in a collision cell. Collisional energy and voltages of the cone for asciminib and voriconazole were optimized as 20/34 and 16/24 V, respectively.

3.2. Specificity

We analyzed six samples of blank plasma and IS, which stands for asciminib. Asciminib, or IS, did not co-elute with any other drugs or endogenous components. The chromatogram shown in Figure 2A is an example of a typical chromatogram of drug-free human plasma (blank) [13,14], which was used in the creation of standards and QC samples. Figure 2B shows blank plasma that has been spiked with IS at a concentration of 85 ng/ml.

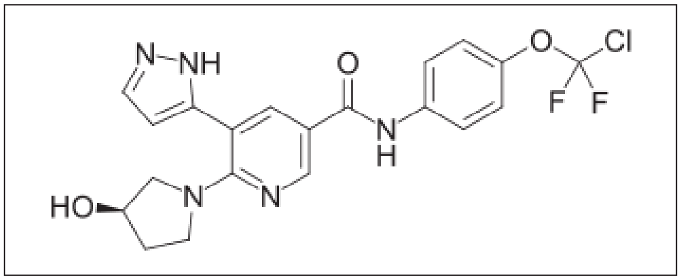

Figure 1. Asciminib chemical structure.

3.3. Calibration curve

The linearity of the test was determined by conducting an analysis on a series of standard blends that included asciminib and IS in human plasma at eight different concentrations ranging from 39 to 1,586 ng/ml. A regression analysis was carried out on the peak height ratios that corresponded to the IS as well as the concentrations [15,16]. For asciminib, the mean equations that were found were $y = 0.0012x - 0.0022$, and the r^2 value was 0.9994 ($n = 6$). It was determined that the calibration curves were appropriate for use by doing a back calculation to determine the concentration of asciminib in human plasma using the calibration curves (Table 1). Every computed concentration was found to be

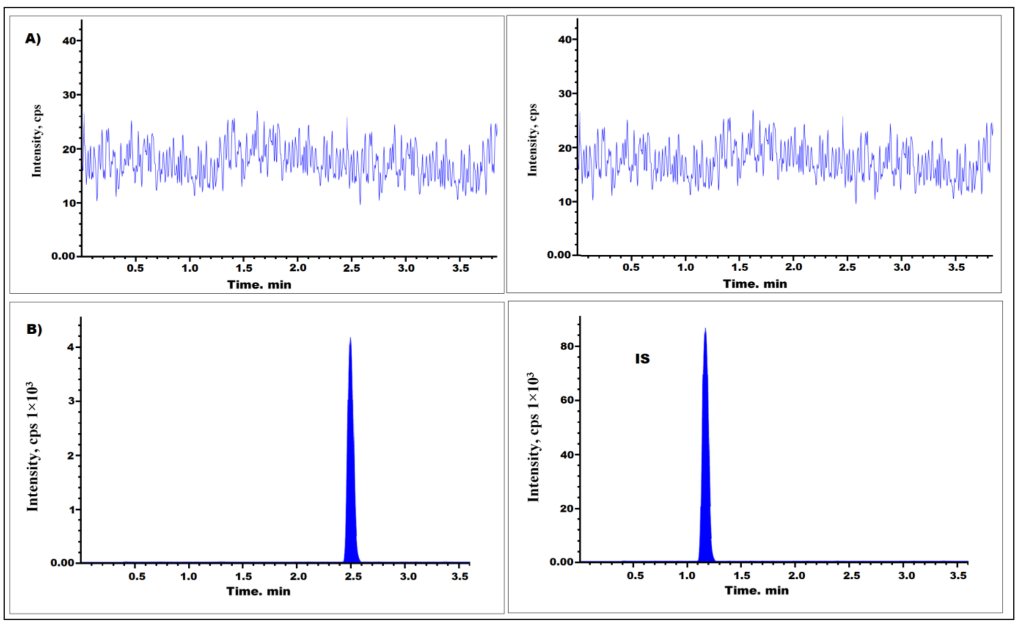

Figure 2. Asciminib chromatogram of (A) blank plasma and (B) LLOQQC levels.

Table 1. Calibration standards for asciminib.

LS-ID	Concentrations (ng/ml)	Average response	IS response	Analyte/IS response
LS-1	39	4,536	95,541	0.047477
LS-2	61	7,194	95,631	0.075227
LS-3	125	13,638	95,174	0.143295
LS-4	300	34,892	95,038	0.367137
LS-5	570	64,995	95,825	0.678268
LS-6	870	104,187	95,289	1.093379
LS-7	1,235	141,640	95,843	1.477834
LS-8	1,586	185,464	95,236	1.947415

LS: Calibration standard.

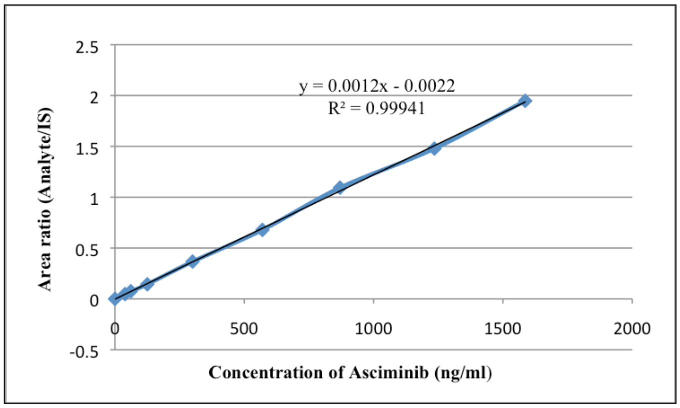

Figure 3. Linearity of asciminib.

substantially below the maximum permissible level. Figure 3 represents the mean calibration curve of asciminib. The Lower limit of quantification quality control (LLOQQC) of asciminib was 39.0 ng/ml (S/N ratio > 10) and was sufficient for accurate quantification of asciminib in the analysis of the plasma sample.

3.4. Accuracy and precision

Accuracy and precision were assessed for four QC levels (39, 111, 793, and 1,189 ng/ml). Intraday precision (n = 10) was ≤4.65% and inter-day precision (n = 20, throughout the course of 3 days) was ≤04.79% for asciminib (Table 2). Interday bias was found to be −4.28% to 5.76% and intraday bias was −3.75% to 4.53 [13]. The LC-MS/MS chromatograms

Table 2. Asciminib precision and accuracy for inter-batch and intra-batch.

Concentration level	Nominal concentration (ng/ml)	Intra-batch			Inter-batch		
		Mean (SD)	%CV	%Bias	Mean (SD)	% CV	% Bias
LLOQ	39	40.77 (1.46)	3.58	4.53	37.33 (1.51)	4.05	−4.28
LQC	111	106.84 (4.92)	4.61	−3.75	116.01 (4.02)	3.47	4.51
MQC	793	824.29 (38.34)	4.65	3.95	767.32 (36.81)	4.79	−3.24
HQC	1,189	1,232.42 (45.41)	3.68	3.65	1,257.49 (41.95)	3.34	5.76

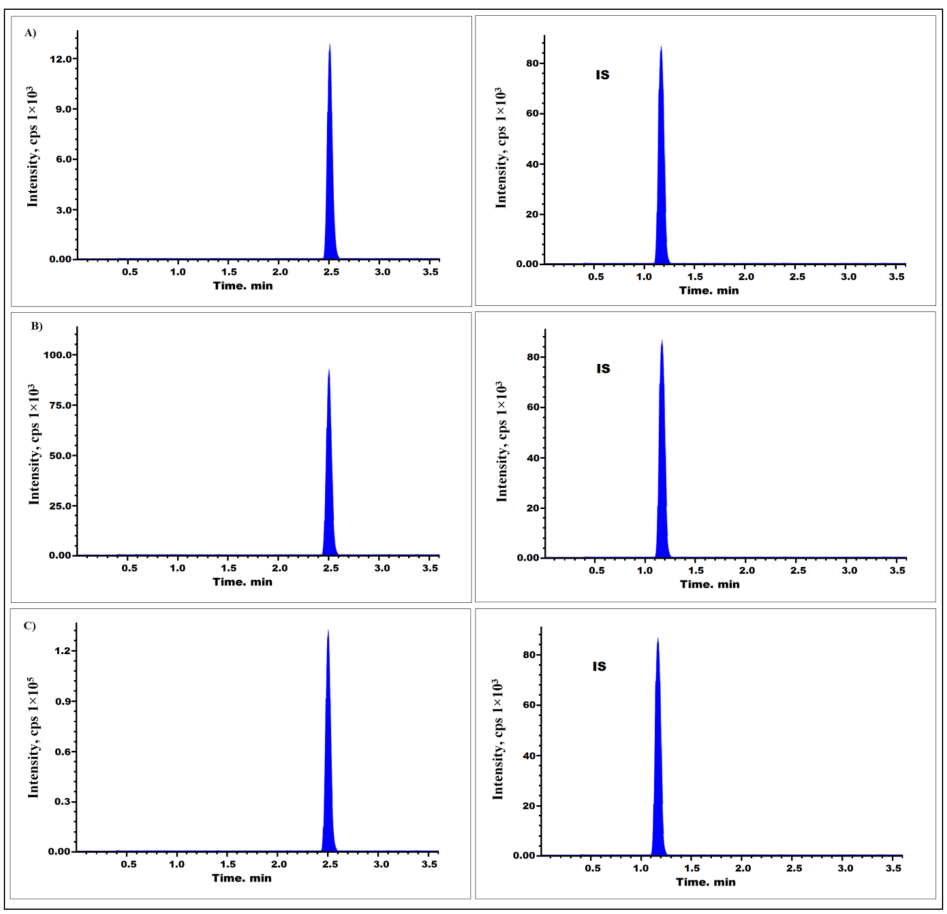

Figure 4. Asciminib chromatograms for (A) LQC, (B) MQC, and (C) HQC level.

Table 3. Recoveries of analytes after the extraction.

Concentration levels	C	D	% Recoveries	% Average recovery	% RSD
LQC	111	106	96.18	99.06	3.26
MQC	793	821	103.57		
HQC	1,189	1,158	97.42		
IS	95,236	93,655	98.34		

C, average recoveries of unextracted solutions; D, average recovery of extracted solutions.

Table 4. Matrix effect of asciminib.

S. No.	LQC			HQC		
	Area of peak without a matrix	Area of peak with a matrix	Matrix factor	Area of peak without a matrix	Area of peak with a matrix	Matrix factor
1	12,910	13,405	103.84	137,935	129,438	93.84
2	12,668	11,956	94.38	139,038	135,493	97.45
3	12,875	12,349	95.92	138,684	143,219	103.27
4	12,802	13,143	102.67	138,951	131,739	94.81
5	12,609	12,151	96.37	139,089	141,787	101.94
6	12,923	12,519	96.88	138,851	131,450	94.67
Mean			98.34			97.66
±SD			3.91			4.04
% CV			3.98			4.13

Table 5. Carry over effect.

Analyte	Asciminib	
Sample ID	Peak area	
	Drug	ISTD
Unextracted samples		
Mobile phase	0	0
Aqueous ULOQ	184,464	95,351
Mobile phase	0	0
Aqueous LLOQ	4,581	95,197
Extracted samples		
STD Blank	0	0
ULOQ	184,509	95,502
STD blank	0	0
LLOQ	4,527	95,327

Table 6. Dilution integrity.

S. No.	Back calculated concentration (ng/ml)	
	1/10th dilution (475 ng/ml)	1/5th dilution (951 ng/ml)
1	463.9014	950.7075
2	465.1803	951.4818
3	463.2147	954.2695
4	473.1803	951.9292
5	467.9529	952.8585
6	465.4979	953.7705
n	6	6
Mean	466.49	952.503
SD	3.34	1.25
% CV	0.716	0.132
% Mean accuracy	98.21	100.16

of low QC (111 g/ml) and high QC (1,189 g/ml) spiked with IS (85 ng/ml) were shown in Figure 4.

3.5. Recovery

Extraction recovery of asciminib at three concentrations (111, 793, and 1,189 ng/ml) and IS at one concentration level (85 ng/ml) were assessed by equating peak responses of spiked solutions before the extraction process and spiked solutions after the extraction process (six sets) [15]. Averagely determined extraction recoveries of asciminib were 99.06% (Fig. 4 and Table 3). Recovery of IS was 98.34%.

3.6. Matrix effects

The matrix effects were quantified by comparing asciminib peak intensity in the absence and presence of matrix constituents. It was measured at low and high QC levels by infusing the six sample solutions. The %CV findings at the LQC and HQC levels were 3.98 and 4.13 (Table 4), respectively.

3.7. Carryover effect

The auto sampler's carryover impact was investigated by injecting mobile phase, aqueous Upper limit of quantification

Table 7. Stability data of asciminib.

Stability study	Concentration level	Comparison samples area Mean	Stability samples area mean	% Mean stability
Short term stability	HQC	138,485	138,119	99.74
	LQC	12,905	12,742	98.73
Long term stability	HQC	138,485	138,157	99.76
	LQC	12,905	12,755	98.84
Freeze thaw stability	HQC	138,485	138,236	99.82
	LQC	12,905	12,703	98.43
Bench top stability	HQC	138,485	138,212	99.80
	LQC	12,905	12,760	98.87
Auto sampler stability	HQC	138,485	138,070	99.70
	LQC	12,905	12,770	98.95
Dry extract stability	HQC	138,485	138,442	99.96
	LQC	12,905	12,754	98.82
Stock solution stability	HQC	138,485	137,770	99.48
	LQC	12,905	12,771	98.96

(ULOQ), Lower limit of quantification (LLOQ), and standard (STD) blank samples. This experiment showed no carryover. The results are summarized in Table 5.

3.8. Dilution integrity

The method's dilution integrity was tested by spiking the plasma with a Dilution quality control (DIQC) concentration of 4,758 ng/ml from a Dilution integrity (DI) spiking solution that was three times the ULOQ. DIQC sample is diluted 1/5 (951 ng/ml) and 1/10 (475 ng/ml). Analyzing samples against calibration curve standards established the precision and accuracy of 1/5 and 1/10 dilution integrity standards. The results are summarized in Table 6.

3.9. Stability

The stability of asciminib and the IS in unprocessed and processed plasma samples at LQC and HQC levels were examined. Table 7 summarizes the findings of the stability investigations.

4. CONCLUSION

The concentration of asciminib in human plasma was determined using a sensitive, accurate, and linear LC-MS/MS approach. C18-cartridges were used to separate plasma samples for the presence of voriconazole, IS, and asciminib. An Atlantis/dC18 (2.1 100 mm, 3 m) stationary phase was used for chromatographic separation. The mobile solvent system was made up of formic acid (0.1%) and methyl alcohol (20:80, v/v). Ionization in the positive method using electrospray ionization allowed for the quantification of the following analytes: asciminib (m/z 450.11/239.09) and voriconazole (IS) (m/z 350.3/281.1). No plasma blank or outside component was found to be interfering. The relationship between asciminib concentration levels and their respective peak response fractions to voriconazole was rectilinear between 39.0 and 1,586.0 ng/ml. Asciminib had a precision of 4.79% both throughout the day and between days. The daily bias ranged from −4.28% to −5.76%, and the hourly bias ranged from −3.75% to 4.53%. Asciminib was recovered at a mean rate of 99.06% during extraction. The recovery of IS was 98.34%. Asciminib in plasma samples can now be routinely quantified using the established method, making it useful for a wide range of applications in industry, forensics, and clinical research. The high sensitivity using the Liquid liquid extraction (LLE) approach was achieved by the development of a straightforward and specific validated LC-MS/MS method for the quantification of infigratinib. The metrics of specificity, sensitivity, carry-over, recovery, precision, accuracy, stability, and the impact of the matrix were used to verify the devised analytical method. Within 6.5 minutes, the drug and IS were eluted on a PhenomenexSB-C18 column (250 × 4.6 mm × 5 m) using acetonitrile and 0.1% V/V formic acid in water at a flow rate of 0.9 ml/minute. Infigratinib's Retention time (RT) was 5.12 minutes, whereas IS's RT was 3.31 minutes. Infigratinib required a total of 6.5 minutes to elute. With a r^2 value of 0.999, the equation for the linear regression line was determined to be $y = 0.994x + 2.662$. The calibration graph %CV measurements for infigratinib came in at 3.73 or below. High QC sample %CV was determined to be 1.64%, and low QC sample %CV was determined to be 0.70%; these numbers represent the matrix effect.

5. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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There is no funding to report.

7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

The study protocol was approved by the Institutional Ethics Committee, (Approval number: FC/New/IND/2022/TF/00992231).

9. DATA AVAILABILITY

All data generated and analyzed are included in this research article.

10. PUBLISHER'S NOTE

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11. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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