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Original Article

BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF ASCIMINIB ANTICANCER DRUG IN BIOLOGICAL MATRICES BY LC–ESI-MS/MS

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ABSTRACT

Objective: A unique liquid chromatography-mass spectrometry technique is essential for determining the concentration of asciminib in biological matrices, and its development is of the utmost importance.

Methods: The samples that were processed were separated using a Reversed Phase-Phenomenex (100 mm x 4.6 mm, 5 µm) C18 analytical column. The column was equipped with an isocratic moveable phase that consisted of 0.1% (v/v) HCOOH and acetonitrile at a ratio of 18:82% (v/v). The flow rate of the phase was 0.70 ml/min. For asciminib, the multiple reaction monitoring mode was used at m/z 450.23/257.3, while for canagliflozin, it was used at m/z 445.13/267.31.

Results: With a correlation coefficient of 0.9998, the method was linear for asciminib throughout the concentration range of 1.0-2100.00 ng/ml. Each day's accuracy percentage relative standard deviation was within 5.74%. For analytes at the low-quality control level, the mean matrix factors ranged from 96.34 to 104.85% with a % Coefficient of Variance (CV) of 4.21; at the high-quality control level, the range was from 94.62 to 103.88% with a %CV of 3.67.

Conclusion: The method that has been developed has the potential to be used to examine the pharmacokinetics and toxicokinetics of asciminib in various biological samples for both forensic and clinical purposes.

Keywords: Asciminib, Cancer, FDA, Sensitivity, Precision, Accuracy

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INTRODUCTION

asciminib is a tyrosine kinase inhibitor used to treat chronic-phase Ph+Chronic Myeloid Leukemia (CML). More precisely, it hinders the activity of the Abelson Kinase (ABL1) in the Breakpoint Cluster Region (BCR)-ABL1 fusion protein, which is responsible for promoting the growth of CML in most affected individuals. It has shown effectiveness in treating Ph+CML with a T315I mutation, which causes resistance to therapy with the mutant BCR-ABL1 compared to the normal BCR-ABL1. The development CML is mostly caused by the Philadelphia chromosomal translocation, which creates a fusion oncogene called BCR ABL1 by combining the ABL and BCR genes. The interaction of this gene leads to the synthesis of a fusion protein, BCR-ABL1, which exhibits transformative properties and elevated tyrosine kinases that promote the growth of CML [1-3].

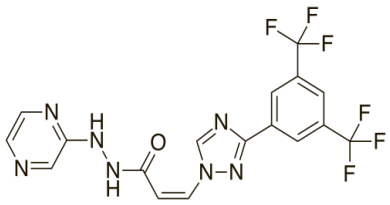

Fig. 1: Chemical structure of asciminib

Asciminib is an allosteric inhibitor that targets the BCR ABL1 tyrosine kinase. By binding to the myristoyl pockets of a specific component of the fusion proteins called ABL1, it has the potential to inhibit the oncogenic activity that would otherwise be triggered by the fusion proteins. This is achieved by immobilizing the fusion proteins in an inactive conformation [4]. The Area Under the concentration-time Curve (AUC_{tau}) and maximum concentration (C_{max}) reached steady state levels at a dosage of 40 mg administered twice daily were 5,262 ng. h/ml and 793 ng/ml, respectively. The chemical is formally known as N-4-[chloro (difluoro) methoxy] phenyl. The chemical compound is-6-[(3R)-3-hydroxy pyrrolidin-1-yl]. The compound is-5-(1H-pyrazol-5-yl). The compound pyridine-3-carboxamide hydrochloride has a chemical formula of $C_{20}H_{18}ClF_2N_5O_3$ and a molar mass of 449.84 g•mol−1.

The literature on asciminib indicates that two analytical methods were used to measure the amount of asciminib: Ultra-Performance Liquid Chromatography (UPLC) [7] and Liquid Chromatography-Mass Spectrometry (LC-MS/MS) [8]. However, there was a need for an analytical technique to accurately measure the amount of asciminib in the biosamples, with a high level of sensitivity and a wider range of concentrations. The present work focuses on developing an LC-MS/MS technique to accurately measure asciminib levels in plasma samples.

MATERIALS AND METHODS

Reagent and chemicals

We acquired canagliflozin from Hetero pharmaceuticals in Hyderabad, India, which has a purity level of 99.52%. In Mumbai, India, Novartis Ltd. supplied the asciminib. The Vivekananda Blood Bank in Hyderabad, India, supplied the drug-free human plasma K2-Ethylenediaminetetraacetic Acid (EDTA) anticoagulant. The study effort used a High-Performance Liquid Chromatography (HPLC)-water purification system developed by Milli-pore in the United States of America, known as MilliQ. We sourced our high-grade ethyl acetate, ammonium acetate, HCOOH, Acetonitrile (ACN), and liquid chromatographic grade methanol from local vendors.

Mass instrument

In order to accomplish Multiple Reaction Monitoring (MRM), the+ve ionization method made use of the electro spray ionization technique. A diluted medication stock solution was injected to improve the operating parameters. The fluxes of nebulizer gas and auxiliary gas were 35 and 50 psi, respectively. An initial temperature of 350 °C was established. Unit resolution was used to monitor Q3 and Q1. Excellent peak intensities were achieved by improvising protonation of analytes when HCOOH was added to the mobile phase. For canagliflozin, the

MRM mode was seen at m/z 445.13/267.31, while for asciminib, it was m/z 450.23/257.3. Using the analyst software 1.5.1 regression line, we were able to estimate the sample concentrations. Here, the peak response ratio technique was used.

Quality control (QC) and calibration standards preparation

The analyte stock solutions were processed in acetonitrile at a concentration of 1000 µg/ml. In order to acquire a concentration range of 1.0-2100.0 ng/ml using acetonitrile, the resultant solution was diluted serially. A mixture of 960 µl of pooled plasma with K2 EDTA and 20 µl of diluted asciminib was used to prepare the calibration standards [9-12]. Twenty microliters of Internal Standard (IS) dilution were added to the final solution after it was produced. A freezer was used to hold solutions with concentrations ranging from 1.0 to 2100.0 ng/ml.

Three different concentrations of quality control standards High-Quality Control (HQC), Median Quality Control (MQC), and Low-Quality Control (LQC) were handled. Following the same procedures as the calibration standard solutions, these QC solutions were adjusted to 1575.00, 1050.00, and 2.8 ng/ml for HQC, MQC, and LQC, respectively. One conical flask was used to process 1 mg/ml stock quantities of canagliflozin in acetonitrile, while the other flask served as an IS. A 1.0 µg/ml solution was obtained by diluting the resultant stock with ACN.

Chromatography

An isocratic mobile phase containing 0.1% v/v HCOOH and ACN at a ratio of 18:82, (% v/v) was used at a flowrate of 0.70 ml/min, and 10 µl** of the sample was injected onto a Reversed Phase-Phenomenex (100 mm × 4.6 mm, 5 µm) C18 analytical column. The whole chromatographic period was 3.0 min, and the analytical column was kept at 45 °C. The chromatographic system used a combination of a Shimadzu HPLC and an Applied Biosystems API-5000 mass spectrometer.

Protocol for sample preparation

Moving 150 µl** of plasma and 100 µl** of canagliflozin (1 µg/ml) into a prelabeled tube and subjecting it to sonication for 10 min was the procedure for executing the drug solution. Centrifugation at 3500 rpm/min for 20 min separated asciminib and canagliflozin from a solution containing 5.0 ml of a solvent system consisting of acetonitrile and ethyl acetate in a 4:3 ratio. The organic component was separated and then dried using a lyophilizer. The dried residue was dissolved in 250 µl** of a mobile solvent and thereafter transferred to LC-vials [13-15]. The LC-MS/MS apparatus was charged with the contents of these vials.

Validation of analytical method

Based on United States Food and Drug Administration (USFDA)-guidelines for sensitivity, specificity, linearity, stability, accuracy, and inter-and intraday precision, the developed LC-MS/MS work was validated [12–14].

RESULTS AND DISCUSSION

Method development

The selective and sensitive LC-MS/MS method needed precise sample cleaning procedures to quantify very low quantities of pharmaceutical formulations in biological sample solutions. The processing of biological materials typically included one of three methods: Solid-Phase Extraction (SPE), Protein Precipitation (PPT), or Liquid-Liquid Extraction (LLE). There is a possibility of matrix component interference, however the PPT technique using organic segments proved straightforward. After that, we gave LLE a go using ethyl acetate and ACN as solvents. As a conclusion, the sample extraction solvent combination of ACN and ethyl acetate (4:3 ratio) produced satisfactory recovery.

Analytical method validation

Selectivity

Eight separate lots of human plasma, including 1-lipemic and 1-hemolytic lots, were used to test the method's selectivity [16]. At the drug and IS retention times, no interference components were found when the blanks' peak areas were compared to the spiked LOQ standard's regions. In fig. 2, the corresponding peaks were shown.

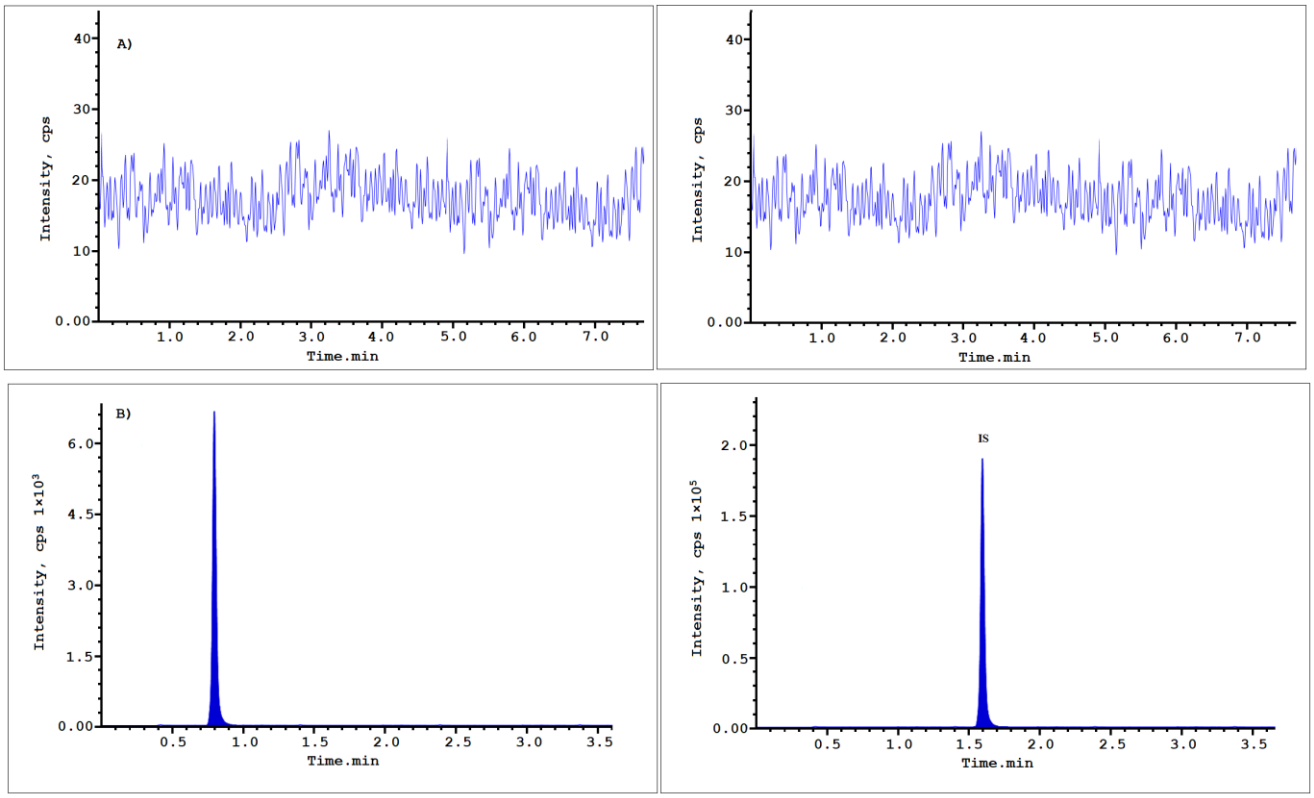

Fig. 2: Chromatograms of asciminib blank plasma (A), spiked LLOQ (B) samples

Recovery

Asciminib and IS recovery values were found to be good and repeatable using this process, demonstrating that it is a robust analytical method [17, 18]. Analyte extraction recoveries were evaluated by comparing peak responses from spiked plasma samples (n = 6) prior to extraction with aqueous sample solution. All QC standards had an average recovery rate of 94.36% (within the specified margin of error). Table 1 (fig. 3) shows the results, which indicate that the IS recovery was 97.69%.

Table 1: Extraction recovery rates of analytes

Concentration level	X	Y	% Recovery	% mean recovery[n]	%RSD
IS	1013246	989837	97.69	94.36	3.14
HQC	1979779	1887523	95.34		
MQC	1319854	1226670	92.94		
LQC	3518	3314.195	94.17		

n=3; X, mean recoveries of unextracted samples; Y, mean recoveries of extracted samples; IS, Internal Standard; HQC, High-Quality Control; RSD, Relative Standard Deviation.

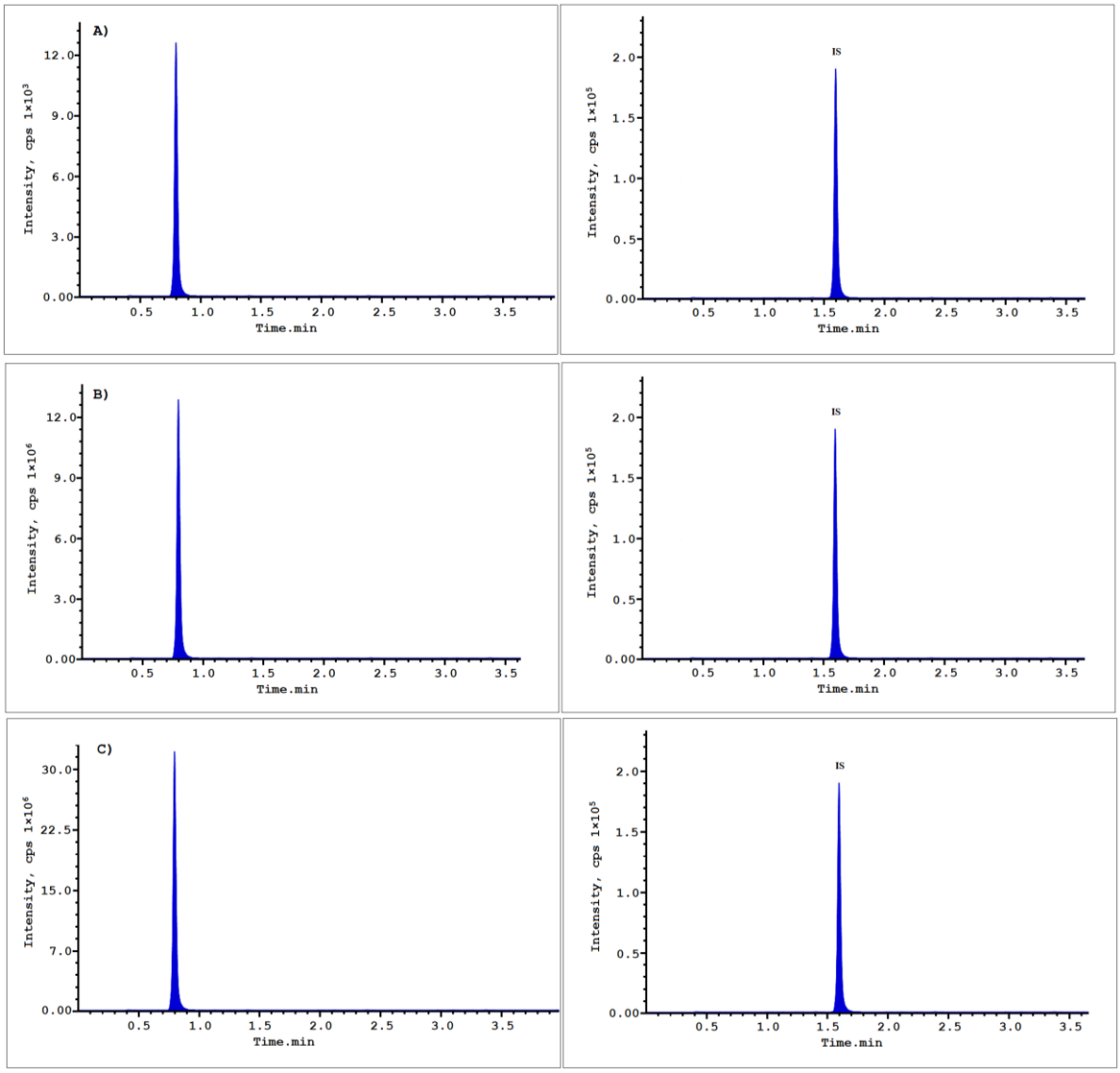

Fig. 3: Chromatograms of asciminib LQC (A), MQC (B) and HQC (C) samples

Linearity and sensitivity

Analyte concentrations between 1.0 and 2100.00 ng/ml were used to produce an eight-point calibration curve. To examine the linear response of the calibration curve, the ratios of the drug's peak responses (y) to IS were plotted against the drug's concentration (x). Equation for linearity was determined to be y = 0.0012x-0.0002, and the correlation coefficient was more than 0.999 (0.9998). In order to get precise results for each calibration standard, the concentration measurements were tested again. The analyte's calibration point's % RSD value ranges were 2.17-4.84, as shown in table 2.

Table 2: Calibration standards for asciminib

CS-ID	Concentration (ng/ml)	Mean[n] (ng/ml)	%RSD	%RE
CS-1	1	0.988	3.62	1.1
CS-2	2.8	2.747	2.97	1.856
CS-3	60	57.588	4.28	4.017
CS-4	260	264.864	3.25	-1.871
CS-5	650	678.443	4.86	-4.376
CS-6	1100	1126.083	2.17	-2.372
CS-7	1575	1524.206	4.84	3.224889
CS-8	2100	2193.893	3.83	-4.473

n=3, CS, Calibration Standards; RSD, Relative Standard Deviation; RE, Relative Error

Accuracy and precision

For all analytical concentrations, the accuracy and precision were tested for both intra-and inter-day groups using six replicate solutions at QC levels (n = 6). The standards used were a HQC control at 1575.00 ng/ml, a middle quality control at 1050.00 ng/ml, a LQC at 2.8 ng/ml, and a Lower Limit of Quantification (LLOQ) at 1.0 ng/ml. Table 3 displays the results for accuracy and precision. The percentage relative error ranged from-1.9 to 4.85% of the nominal values, while the intraday and interday precision %RSDs were within 5.74%.

Table 3: Intra-batch and inter-batch precision and accuracy

Concentration level	Nominal concentration (ng/ml)	Intra-batch			Inter-batch		
		Amount found (ng/ml)	%RSD	%RE	Amount found (ng/ml)	%RSD	%RE
HQC	1575	1647.221	4.91	4.58	1651.327	5.74	4.85
MQC	1050	1091.412	2.85	3.94	1089.435	3.82	3.76
LQC	2.8	2.903	4.31	3.68	2.921	2.17	4.32
LLOQ	1	0.981	3.56	-1.9	0.971	3.64	-2.9

HQC, High-Quality Control; MQC, Median Quality Control, LQC, Low-Quality Control; LLOQ, Lower Limit of Quantification; RSD, Relative Standard Deviation; RE, Relative Error

Matrix effect

Eight different lots, including lipemic-1 and hemolytic-1, were used to collect blank plasma samples in order to evaluate this parameter. Each batch was processed according to the sample preparation technique after 100 microliters of blank plasma was collected. Processing was carried out at either the LQC or HQC levels for both the aqueous and post-extracted solutions [19-22]. A formula was used to determine the matrix effect, which is defined as the percentage of the aqueous sample's peak response divided by the peak response of the postextraction samples, multiplied by 100. Table 4 shows that for analytes at the LQC level, the mean matrix factor ranged from 96.31% to 104.72% with a %CV of 4.57, whereas for analytes at the HQC level, it was 94.63% to 103.87% with a %CV of 3.71.

Table 4: Matrix factor for asciminib

S. No.	HQC			LQC		
	Concentration in absence of matrix	Concentration in presence of matrix	Matrix factor	Concentration in absence of matrix	Concentration in presence of matrix	Matrix factor
1	1581.61	1506.33	95.24	2.678	2.542	94.89
2	1563.57	1495.87	95.67	2.640	2.574	97.52
3	1557.78	1594.26	102.34	2.750	2.648	96.31
4	1465.49	1522.34	103.87	3.004	2.833	94.25
5	1453.24	1416.62	97.48	2.67	2.795	104.72
6	1610.87	1524.23	94.63	2.771	2.873	103.65
Mean			98.20			98.58
± SD			3.60			4.15
% CV			3.71			4.57

HQC, High-Quality Control; LQC, Low-Quality Control; SD, Standard Deviation; CV, Coefficient of Variation

Dilution integrity

We made six separate copies of the sample solutions and compared them to a set of newly spiked calibration solutions to get an approximation. Researchers found that diluting the medication with plasma blank increased the upper concentration limit to 6,000 ng/ml [20]. With a coefficient of variation for the analyte below 4.09, the average back-calculated concentrations for 2-fold and 4-fold diluted sample solutions ranged from 95.78 to 103.52 percent.

Carryover effect

Use of sample solutions in the following order: lower limit of quantification quality control LLOQ QC of drug, plasma blank, and Upper Limit of Quantitation (ULOQ) of drug and plasma blank allowed for the processing of this parameter. During the analysis, no drug or IS carryover was found [21].

Stability studies

Both aqueous and matrix-based samples were subjected to stability testing. Both the long-term and short-term stability assessments of aqueous samples were assessed in the following ways:

Aqueous solution stability

The MQC standard solutions were processed and kept at 25.0 °C for 24 h to ensure short-term stability. Using a newly processed MQC

standard sample solution and an injection of six duplicates of these standards at 25.0 °C, we evaluated any variations. At 94.95%, stability was the average. The MQC standard solutions were processed to ensure long-term stability, and the product was kept at 2-8 °C for 40 days. For the purpose of estimating any discrepancies with the newly processed MQC standard sample solution, six sets of these standards were injected. At 96.74%, stability was the average.

Plasma stability in humans

Using LQC and MQC standards processed in plasma solution, which were collected at-20.0 °C and maintained at 25.0 °C (room temperature) for seven hours, benchtop stability was achieved. For the purpose of estimating discrepancies with newly processed LQC and MQC standard sample solutions, six copies of these standards were inserted [17]. Between 96.34% and 95.31% of the time, stability was evident. After 22 h of processing in an auto sampler at 10.0 °C, the in-injector stability was determined to be 95.34-103.85% using LQC and MQC standards in plasma solution. Plasma solutions were tested for freeze-thaw stability using LQC and MQC standards. The expected percentage of recovery, after four freeze-thaw cycles, was determined to be between 98% and 103.85%. Results showed a wet extract stability of 94.87–93.71% when LQC and MQC standards were processed in a plasma solution at 25.0 °C for 7 h (table 5).

Table 5: Stability results of asciminib

Parameter	QC level	N	M	%RSD	%Stability
Wet extract stability	LQC	2.8	2.763	4.63	98.69
	HQC	1575	1615.005	3.87	102.54
Freeze–thaw stability	LQC	2.8	2.656	3.94	94.87
	HQC	1575	1475.933	4.07	93.71
Benchtop stability	LQC	2.8	2.698	3.81	96.34
	HQC	1575	1498.928	2.93	95.17
In-injector stability	LQC	2.8	2.669	2.93	95.34
	HQC	1575	1635.638	2.51	103.85

N, nominal concentration (ng/ml); M, mean concentrations (ng/ml) of analytes, HQC, High-Quality Control; LQC, Low-Quality Control; QC, Quality Control; RSD, Relative Standard Deviation

Extended accuracy and precision run

Forty sets of HQC and LQC, together with one batch of calibration curve standards, were prepared and estimated before the extended accuracy and precision run was conducted [19]. Table 6 displays the results pertaining to the precision and accuracy that were expanded. In terms of accuracy and stability, asciminib performed well at both the LQC and HQC levels, with scores of 3.89 and 95.34%, respectively.

Table 6: Extended precision and accuracy analysis

Analyte	Conc. level	A	B	%RSD	%Stability
asciminib	HQC	1575	1635.282	4.12	103.74
	LQC	2.8	2.671	3.89	95.41

A, Original concentration (ng/ml); B, mean of 6 concentrations (ng/ml), HQC, High-Quality Control; LQC, Low-Quality Control; RSD, Relative Standard Deviation

CONCLUSION

An LC–MS/MS method that is specific, accurate, and sensitive was developed and validated in a short amount of time for the purpose of determining the amount of asciminib present in human plasma. For the purpose of achieving consistent recovery results for both the drug and the IS, the LLE extraction technique was used in the method that was designed. The approach was validated in compliance with the criteria provided by the FDA across the concentration range of 1.0–2100.00 ng/ml for asciminib, and the correlation coefficient value was found to be 0.9998. Both the intraday and interday(RSDs were within 5.74% of the nominal values, and the rate of relative error ranged from-1.9 to 4.85 percent. At the LQC level, the mean matrix factor was found to be between 96.34 and 104.85%, with a % CV of 4.21. At the HQC level, the matrix factor range was 94.62 to 103.88%, with a %CV of 3.67. Because of this, the method that was created has the potential to be used for the purpose of effectively studying pharmacokinetics and toxicokinetics in forensic and clinical studies of asciminib in a variety of biological materials.

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Nil

AUTHORS CONTRIBUTIONS

Both the authors have contributed in design, experiment and execution of the work.

CONFLICT OF INTERESTS

Declared none

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