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Bio-Analytical Method Development and Validation of Asciminib and its Application to Pharmacokinetic Studies in Rat Plasma by Using RP-HPLC

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The aim of the proposed study was to validate a rapid, uncomplicated, accurate, robust and sensitive bio-analytical method for quantifying asciminib using ivosidenib as an internal standard in rat plasma. This work provides a summary of recent advances in bio-analytical HPLC methodologies employing a Luna phenyl hexyl column (150 × 4.6 mm, 3.5) with an organic mobile phase of potassium dihydrogen phosphate (adjusted to pH 3.0 with orthophosphoric acid) and acetonitrile in the ratio 40:60. With a correlation coefficient of 0.999, the asciminib calibration curve was linear over the range of 100-2000 ng/mL. Using liquid-liquid extraction, the percent of recovered ASB ranged between 97.8 and 99.9%. The plasma stability of the bench-top and auto sampler remained stable for 24 h in the auto sampler. The $T_{1/2}$ is 5 h and T_{max} is 3 h, as calculated from the pharmacokinetic parameters. The application indicates that all parameters of system suitability, specificity, linearity and accuracy are in good agreement with the prescribed limits; consequently, the current method is highly stable, rapid and capable of analyzing plasma samples. This method is ideally adapted for determining asciminib in pure or dose form due to its novel technology.

Keywords: Asciminib, Validation, HPLC, Rat plasma, Pharmacokinetics.

INTRODUCTION

Asciminib is a small molecule drug developed by Novartis for the treatment of chronic myeloid leukaemia [1,2], which has been approved by the US FDA in 2021. It is also known as Scemblix, which is N-[4-(chloro-difluoro-ethoxy)phenyl]-6-[(3*R*)-3-hydroxy-pyrrolidin-1-yl]-5-(1*H*-pyrazol-3-yl)pyridine-3-carboxamide hydrochloride with the chemical formula $C_{20}H_{18}N_5O_3ClF_4 \cdot HCl$ [3]. It works by selectively targeting and inhibiting a specific mutant form of the BCR-ABL1 protein [4], which is the cause of chronic myeloid leukaemia. Asciminib is unique among chronic myeloid leukaemia treatments in that it targets a different part of the BCR-ABL1 protein than other tyrosine kinase inhibitors like imatinib, nilotinib and dasatinib [4]. This means that asciminib can be effective in patients who have developed resistance to other tyrosine kinase inhibitors. Clinical trials have shown that asciminib is effective in treating chronic myeloid leukaemia [5,6], including in patients who have developed resistance to other tyrosine kinase inhibitors. It has also been found to have a favourable safety profile, with fewer side effects compared to other tyrosine kinase inhibitors [7,8]. Asciminib represents a promising new treatment option for chronic myeloid leukaemia patients, particularly those who have developed resistance to other tyrosine kinase inhibitors. However, more research is needed to fully understand its efficacy and safety profile and to determine its optimal use in clinical practice. Bioanalytical method development using HPLC [9-13], is significant in determining the concentration of drugs and their metabolites in biological samples. It ensures the safety and efficacy of drugs by providing accurate and precise measurements, facilitating drug development and enabling pharmacokinetic studies. Only one study has been published on the determination of asciminib by UHPLC till now. In the study by Priya *et al*. [14], asciminib was separated proficiently using C18 column operated with mobile phase trifluoro acetic acid in water (0.1%) and acetonitrile in the ratio of 75:25 v/v achieved retention time of 0.925 min and linearity of 0.999.

Till today there is no established bioanalytical method for the determination of asciminib in various biological matrices.

The author aimed to achieve a simple, novel, bioanalytical method using internal standard to overcome the system or method errors. The proposed method provides accurate and sensitive quantification of asciminib with ivosidenib (IVB) as an internal standard which can be used in pharmacokinetic studies and therapeutic drug monitoring in patients receiving asciminib therapy.

EXPERIMENTAL

Merck (India) Ltd. in Worli, Mumbai, India, supplied the HPLC-grade acetonitrile, KH_2PO_4 and water. Zydus Cadila in Ahmadabad supplied the active pharmaceutical ingredient (API) of asciminib used as the reference standard and Bio Needs in Bangalore supplied the plasma used as the internal standard.

Selection of animals for pharmacokinetic study: In order to conduct *in vivo* pharmacokinetic investigations, Biological E Limited in Hyderabad, India provided the researchers with six healthy rats weighing between 200 and 250 g each. The institute's animal ethics committee gave their stamp of approval to the protocol for the study that used animals (Reg. No. 1074/PO/Re/S/05/CPCSEA).

Chromatographic conditions: The Luna phenyl hexyl column (150 × 4.6 mm, 3.5 µ) was used for chromatographic separation. As a mobile phase, a combination of KH_2PO_4 adjusted to pH of 3.0 with orthophosphoric acid and acetonitrile in a 40:60 v/v ratio was provided at a flow rate of 1.0 mL/min at room temperature. The injection volume was 10 µL and the duration of operation was 7 min. Using a 0.45 µm membrane filter paper, the final solution was filtered.

For bioanalytical examination of the studied drug and subsequent data processing, a Waters alliance e-2695 HPLC chromatographic system with UV/Vis dual absorbance detector 2487, with an automated sample injector, integrated with Empower-2.0 software was applied. Sartorius electronic balance was employed.

Selection of wavelength: By scanning between 200 and 400 nm, ASB UV spectrum was obtained. As depicted in Fig. 1, the greatest absorbance for the selected drug was measured at 258 nm, which was selected from the maximum wavelength.

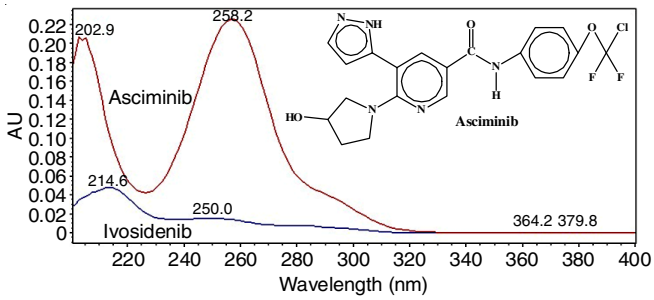

Fig. 1. Superimposed UV spectra of asciminib and ivosidenib

Preparation of stock solution: The mobile phase may be used as a diluent. Weighed and transferred 8 mg of asciminib and ivosidenib working standards into a clean, dry, 10 mL volumetric flask. Fill the flask to the right volume with diluent. With this pipette, transfer and dilute 0.5 mL into a 10 mL volumetric flask. Likewise, transfer 1 mL of aforementioned solution to a second 10 mL volumetric flask and dilute to a final concentration of 4000 ng/mL using the same diluent.

Extraction method and calibration solution preparation: Added 50, 125, 250, 375, 500, 625, 750 and 1000 µL of stock asciminib solution to 200 µL of plasma in eight labeled micro-centrifuge tubes with a capacity of 2000 µL to get concentrations of 100, 250, 500, 750, 1000, 1250, 1500 and 2000 ng/mL of stock asciminib solution. Vortex all samples for 1 min. Thereafter, 500 µL of IVB (1000 ng/mL) were added to each concentration solution as an internal standard. All generated solutions were repeatedly vortexed for 1 min at a medium speed. In addition, 2000, 1300, 1250, 1175, 1050, 925, 800, 675, 550 and 300 µL of acetonitrile were added to each labeled tube and the resulting solution, which had a final volume of 2000 µL, was vortexed at medium speed for 2 min. All samples were centrifuged at 5000 rpm for 15 min and the supernatant was filtered through 0.45 µm filter paper. Finally, 20 µL of each sample were evaluated under specific chromatographic conditions.

Method: In order to quantify asciminib in rat plasma using a precise, accurate and reproducible bioanalytical reverse phase HPLC-UV method, various parameters were evaluated in accordance with industry standards. The above optimized conditions with respect to detection wavelength, mobile phase, stationary phase, extraction techniques however were found to be suitable for the quantitative determination of asciminib.

Linearity: Using eight concentrations of asciminib solution ranging from 100 to 2000 ng mL^{-1}, the linearity was confirmed. Using the aforementioned extraction method, the linearity experiment was conducted to determine the linearity of detector's response to various concentrations of asciminib solutions spiked in rat plasma, with a fixed concentration of IVB solution used as an internal standard. The prepared concentrations were then injected into the HPLC system and calibration curves were constructed by plotting peak areas of asciminib *versus* concentrations of asciminib. The correlation coefficient, slope, intercept, LOD and LOQ were determined using regression equations.

Precision and accuracy: Six injections of each concentration at the LLOQ (100 ng mL^{-1}), LQC (500 ng mL^{-1}), MQC (1000 ng mL^{-1}) and HQC (1500 ng mL^{-1}) quality control levels were evaluated for intraday precision on the intra-day and for inter-day precision on distinct days. For precision analysis, %RSD was computed using the obtained area response ratio values. By comparing the measured response to the true response at LLOQ (100 ng mL^{-1}), LQC (500 ng mL^{-1}), MQC (1000 ng mL^{-1}) and HQC (1500 ng mL^{-1}) quality control levels, intraday and inter-day percentages of accuracy were also determined, which should be 20% at the LLOQ level and 15% at the HQC, MQC and LQC levels.

% Recovery: Recovery of asciminib can be accomplished by comparing the analytical responses for extracted samples at three concentrations with non-extracted standards that represent a 100% recovery rate. The analyte's recovery need not be 100%. Six replicate injections of extracted plasma samples and corresponding extracts of blanks spiked with the analyte post-extraction were injected at three QC levels: low quality control (LQC), medium quality control (MQC) and highest quality

control (HQC) with corresponding concentrations of 100, 500, 1000 and 1500 ng mL^{-1} and the recovery of asciminib may be determined by comparing the analytical responses of both.

Specificity: Analyzing six different rat plasma samples allowed for a selectivity test to be carried out. This test was designed to determine whether or not there were any interferences with the asciminib and ivosidenib retention times.

System suitability test: To verify analytical performance and reproducibility of chromatographic conditions, system suitability parameters such as retention time, tailing factor, theoretical plates, resolution and asymmetric factor were calculated and the obtained results were compared with standard values, further evaluated in order to calculate the % RSD values.

Test of stability: Six replicates were used at each concentration level to conduct stability experiments in plasma at the LQC, MQC and HQC concentrations. According to US Food and Drug Administration guidelines, an analyte is considered stable if its change is less than 15%. For bench-top stability studies, the QC samples at the above-mentioned concentration that were stored at room temperature for 24 h were compared to the plasma extract samples that were injected promptly. The freeze-thaw stability was determined by comparing samples that had been frozen at -31 ºC and thawed three times with freshly injected internal control samples at the conclusion of the third cycle. In order to determine the short-term stability of LQC, MQC and HQC samples, six replicates of each sample were stored at 7 ºC for seven days, followed by HPLC analysis. Six replicates per level of LQC, MQC and HQC must be conducted at 7 ºC at regular intervals beginning with 1, 7, 14, 21 and 28 days. For auto-sampler stability, three samples with the aforementioned QC levels were prepared and stored in the auto-sampler at 15 ºC for 0 to 24 h and accuracy was determined every hour at each concentration level. We compared the stability of dry and moist extract plasma samples that were injected after storage at room temperature for 12 h and 18 h at 2-8 ºC to that of freshly extracted samples. The sample is considered stable if its relative standard deviation is less than 15%.

Matrix effect: Different lots of human plasma were spiked with analyte at LQC and HQC levels to determine the matrix effect. At each level, tests were performed in duplicate. The acceptance criterion for each back-calculated concentration was a 15% deviation from the nominal value as specified by the guidelines.

Carry forward effect: The carry-over effect is examined by placing blank samples after the standard at the ULOQ. The blank sample response should be less than the LLOQ.

Application to bioavailability studies: One night prior to the experiments, all animals were deprived of food and water. A local anesthetic was employed. A pharmacokinetic investigation was conducted on the asciminib formulation. When the samples were administered, the rodents were fasting. Following oral administration of asciminib, blood samples were obtained from the marginal ear vein of rats at 1, 2, 3, 5, 6, 7, 8 and 9 h using a 25-gauge, 5/8-inch cannula and a volume of 0.5 mL to 1.0 mL. Blood was collected in Eppendorf tubes containing an EDTA solution comprising 10%. At 2-8 ºC, blood was centrifuged for 30 min at 5000 rpm and 2-8 ºC. The clear plasma precipitate was collected and stored at -30 ºC prior to analysis. Using a newly developed technique, plasma samples were subjected to liquid-liquid phase extraction and drug content analysis. Following the present study, the animals were returned to the animal house for rehabilitation. Plasma concentration information was utilized to examine the pharmacokinetic parameters of oral asciminib administration. On the basis of the data, pharmacokinetic parameters including AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max} (the time at which C_{max} occurred), $T_{1/2}$, C_0, V_d, Cl and Ke were calculated. The concentration-time curve was measured from zero to infinity using the trapezoidal rule. Each value is represented by its mean and standard deviation.

RESULTS AND DISCUSSION

The developed bioanalytical method was validated according to Food and Drug Administration (FDA) regulations.

Linearity: The calibration standard exhibited a proportional relationship between peak area and concentration. Asciminib concentrations range from 100 to 2000 ng/mL and the correlation coefficients are 0.999, whereas the intercept and slope are 0.00576 and 0.00148, respectively. LOD and LOQ were calculated to be 86.5 and 262.1 ng mL^{-1} respectively. Table-1 and Fig. 2 display the related chromatograms of asciminib.

TABLE-1
RESULTS OF LINEARITY FOR ASCIMINIB

Conc. (ng/mL)	Area response ratio	Conc. (ng/mL)	Area response ratio
100	0.101	2000	1.978
250	0.252	Slope	0.001
500	0.483	Intercept	0.0032
750	0.755	CC	0.9996
1250	0.998	LOD	86.5 ng mL^{-1}
1000	1.209	LOQ	262.1 ng mL^{-1}
1500	1.467		

Precision and accuracy: As shown in Table-2, both the accuracy and precision of the results obtained were within the permissible limits. Mean area response ratios at the LLOQ (100 ng mL^{-1}), LQC (500 ng mL^{-1}), MQC (1000 ng mL^{-1}) and HQC (1500 ng mL^{-1}) quality control levels were 0.099, 0.488, 1.00 and 1.468, respectively and the intraday precision for the aforementioned concentrations of asciminib was 0.09, 0.05, 0.02 and 0.01. It was determined that the intraday accuracy percentage for the aforementioned concentrations was 99.3, 99.4, 99.8 and 100.3 for 100, 500, 1000 and 1500 ng mL^{-1} asciminib concentrations, respectively. Similarly, for the inter-day precision study, the mean area response ratios at the LLOQ (100 ng mL^{-1}), LQC (500 ng mL^{-1}), MQC (1000 ng mL^{-1}) and HQC (1500 ng mL^{-1}) quality control levels were 0.089, 0.456, 0.99 and 1.562, respectively with a relative standard deviation of 0.09, 0.10, 0.03 and 0.02 for the four quality control levels. Moreover, the inter-day accuracy percentage for the same was calculated to be 100.14, 100.06, 100.01 and 100.02.

Recovery: In samples of HQC, MQC and LQC, the percentage recoveries and percent CV of asciminib are determined by comparing the height areas of extracted and unextracted standards. The percentage recoveries for asciminib were found to be 99.87

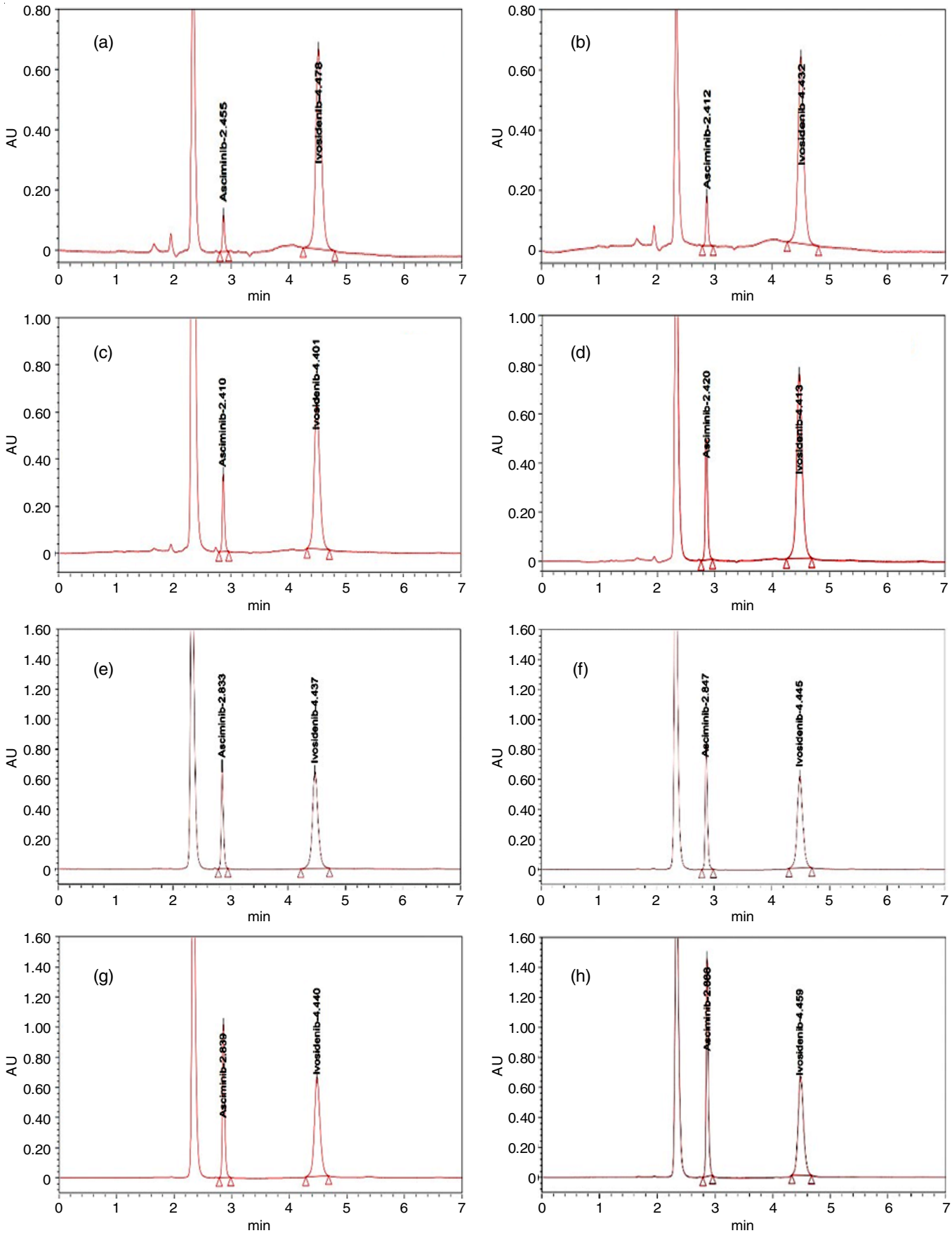

Fig. 2. Demonstration of linearity of plasma spiked asciminib concentrations of 100, 250, 500, 750, 1000, 1250, 1500 and 2000 ng/mL represented by chromatograms a, b, c, d, e, f, g and h, respectively

TABLE-2
PRECISION AND ACCURACY OF ASCIMINIB

QC name	Intra-day precision				Inter-day precision			
	LLQC (100 ng/mL)	LQC (500 ng/mL)	MQC (1000 ng/mL)	HQC (1500 ng/mL)	LLQC (100 ng/mL)	LQC (500 ng/mL)	MQC (1000 ng/mL)	HQC (1500 ng/mL)
QC sample-1	100.18	500.19	1000.16	1500.12	100.14	499.48	1000.65	1500.23
QC sample-2	100.01	500.65	1000.12	1500.24	100.05	500.18	1000.23	1500.45
QC sample-3	100.06	500.35	1000.14	1500.35	100.06	500.21	1000.11	1500.22
QC sample-4	100.27	500.48	1000.25	1500.45	100.19	500.78	999.97	1500.68
QC sample-5	100.12	500.86	1000.56	1500.56	100.28	500.59	1000.01	1499.87
QC sample-6	100.15	500.58	1000.68	1500.13	100.11	500.66	999.89	1500.01
Mean of ARR	0.099	0.488	1.00	1.468	0.089	0.456	0.99	1.562
Mean* ± SD	100.13±0.09	500.52±0.230	1000.32±0.24	1500.31±0.17	100.14±0.09	500.32±0.48	1000.14±0.27	1500.24±0.29
%CV	0.09	0.488	0.02	1.468	0.09	0.10	0.03	0.02
Accuracy	99.3%	99.4%	99.8%	100.3%	100.14%	100.06%	100.01%	100.02%

*Mean n = 6 determinations; ARR is Area response ratio

99.95 and 99.98% and the coefficient of variation (%CV) was within acceptable limits as shown in Table-3, indicating that the bioanalytical technique had a high extraction rate.

Specificity: The chromatograms of rat plasma that did not contain any additives or standards did not show any interference peaks in the blank at the appropriate retention durations of asciminib and ivosidenib as shown in Fig. 3.

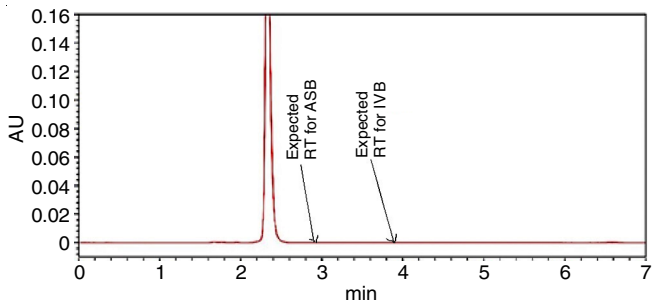

Fig. 3. Typical chromatogram of blank rat plasma

Matrix effect: For matrix effect, the mean percent accuracy of three replicate injections at LQC and HQC quality control levels of asciminib samples that were prepared with six distinct plasma lots was determined to be 101.1% and 100%, respectively and is within the acceptable range of 80-120%. The medication's % CV was 0.01 at the LQC level but just 0.006 at the HQC level. This indicates that the effect of the matrix on the ionization of the analyte is within the permissible range, as summarized in Table-4.

Stability: In a battery of stability tests, the concentrations of asciminib samples were within 15% of those of fresh samples. Through a bench-top stability study, the accuracy of asciminib sample was determined to be 100.95, 99.48 and 99.95, whereas the accuracy for freeze-thaw stability was 100.99, 100.02 and

TABLE-4
MATRIX EFFECT OF ASCIMINIB

Plasma lots	LQC	HQC
	Mean area ± SD (n = 3)	Mean area ± SD
LOT-1	28546 ± 2.0	85327 ± 6.0
LOT-2	28555 ± 2.5	85344 ± 4.5
LOT-3	28564 ± 2.5	85371 ± 4.6
LOT-4	28572 ± 3.5	85356 ± 1.7
LOT-5	28591 ± 4.0	85385 ± 4.5
LOT-6	28581 ± 3.0	85401 ± 9.6
Mean ± %RSD	0.01	0.006
Mean % accuracy	101.12	100

100.01 at the LQC, MQC and HQC levels, respectively. The results of short-term and long-term stability tests, as indicated by the % CV within 15% of the accepted limit value, indicate that the stability of the samples is up to 24 days. The auto-sampler stability results of the proposed method, which were 101.01, 100.06 and 100.05, demonstrate the stability of the processed sample in the auto-sampler in comparison to fresh samples. The overall stability results were found to be within the assay variability limits throughout the entire proce-dure as shown in Table-5.

System suitability: Fig. 4 depicts a typical chromatogram of asciminib and the internal standard ivosidenib, with retention times of 2.8 and 4.4 min and a resolution of 5.32. For asciminib and ivosidenib, respectively, the number of calculated theoretical plates was 6949 and 7123 and the tailing factors were 1.05 and 0.85, respectively. Table-6 provides a summary of these findings.

Investigation of pharmacokinetics: The aforementioned technique was utilized to investigate the pharmacokinetic parameters of six rodents following oral administration of asciminib and the internal standard IVB. On the other hand, pharmaco-

TABLE-3
RECOVERY STUDIES OF ASCIMINIB

Quality control level	Extracted			Unextracted		
	LQC	MQC	HQC	LQC	MQC	HQC
Mean area* ± SD	28610 ± 4.8	58054 ± 7.7	85419 ± 4.4	28625 ± 4.2	58140 ± 9.5	85437 ± 7.2
%CV	0.017	0.013	0.005	0.015	0.016	0.008
Mean % recovery	99.95	99.87	99.98			

*Mean n = 6 determinations

TABLE-5
STABILITY RESULTS OF ASCIMINIB

Stability type	QC level	Mean of measured concentration (ng/mL) ± SD, n = 6	CV (%)	Accuracy (%)
Bench top stability (room temperature, 4-24 h)	LQC (500 ng/mL)	504.74 ± 0.10	0.02	100.95
	MQC (1000 ng/mL)	994.80 ± 0.18	0.02	99.48
	HQC (1500 ng/mL)	1499.31 ± 0.11	0.01	99.95
Freeze thaw stability (frozen at –31 °C thawed 3 times)	LQC (500 ng/mL)	504.94 ± 0.09	0.02	100.99
	MQC (1000 ng/mL)	1000.21 ± 0.15	0.02	100.02
	HQC (1500 ng/mL)	1500.17 ± 0.11	0.01	100.01
Short term stability (7 days at 7 °C)	LQC (500 ng/mL)	496.98 ± 1.02	0.20	99.40
	MQC (1000 ng/mL)	989.09 ± 4.46	0.45	98.91
	HQC (1500 ng/mL)	1494.83 ± 0.81	0.05	99.66
Long term stability (after 28 days)	LQC (500 ng/mL)	492.01 ± 3.79	0.77	98.40
	MQC (1000 ng/mL)	985.11 ± 4.78	0.48	98.50
	HQC (1500 ng/mL)	1494.61 ± 2.0	0.13	99.60
Auto sampler stability	LQC (500 ng/mL)	505.06 ± 0.19	0.04	101.01
	MQC (1000 ng/mL)	1000.67 ± 0.42	0.04	100.06
	HQC (1500 ng/mL)	1500.76 ± 0.50	0.03	100.05
Wet extract stability (12 h)	LQC (500 ng/mL)	505.0588 ± 0.10	0.009	101.01
	MQC (1000 ng/mL)	999.89 ± 0.08	0.010	99.98
	HQC (1500 ng/mL)	1500.23 ± 0.11	0.008	100.01
Wet extract stability (18 h)	LQC (500 ng/mL)	505.40 ± 0.11	0.021	101.08
	MQC (1000 ng/mL)	999.44 ± 0.09	0.010	99.94
	HQC (1500 ng/mL)	1500.57 ± 0.11	0.008	100.03
Dry extract stability (12 h)	LQC (500 ng/mL)	505.61 ± 0.09	0.02	100.12
	MQC (1000 ng/mL)	1000.42 ± 0.07	0.01	100.04
	HQC (1500 ng/mL)	1500.93 ± 0.09	0.01	100.06
Dry extract stability (18 h)	LQC (500 ng/mL)	506.31 ± 0.10	0.02	100.26
	MQC (1000 ng/mL)	1000.69 ± 0.07	0.01	100.07
	HQC (1500 ng/mL)	1500.93 ± 0.11	0.01	100.08

TABLE-6
SUMMARY OF STUDIES ON SYSTEM SUITABILITY

	Peak area		Retention time (Rt)		Theoretical plates (N)		Tailing factor (T)		Resolution (Rs)
	Asciminib	Ivosidenib	Asciminib	Ivosidenib	Asciminib	Ivosidenib	Asciminib	Ivosidenib	
STD-1	58222	58235	2.84	4.43	6952	7142	1.13	0.97	5.30
STD-2	58228	58239	2.84	4.43	6956	7147	1.15	0.96	5.36
STD-3	58236	58243	2.84	4.43	6959	7153	1.18	0.95	5.38
STD-4	58239	58247	2.85	4.44	6963	7157	1.19	0.94	5.40
STD-5	58245	58249	2.85	4.44	6965	7159	1.22	0.93	5.44
STD-6	58250	58255	2.86	4.45	6969	7165	1.25	0.91	5.27
Mean*	58237	58245	2.84	4.44	6961	7154	1.19	0.94	5.40
± SD	10.42	7.20	0.01	0.01	6.22	8.35	0.04	0.02	0.06
%CV	0.02	0.01	0.27	0.21	0.09	0.12	3.72	2.29	1.18

*Mean n = 6 determinations

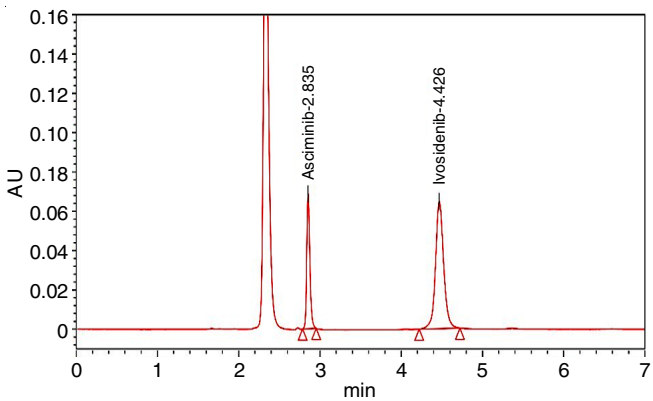

Fig. 4. A chromatogram depicting the elution of asciminib and ivosidenib under optimized HPLC conditions

kinetic characteristics were determined using a model of non-compartmental timing analysis. At intervals of 0, 1, 2, 3, 4, 6, 7, 8 and 9 h, the concentration of asciminib in plasma was measured and the mean plasma concentration–time profile profiles are depicted in Fig. 5. Based on the concentration–time curves, the maximum plasma concentration (C_{max}) and time to attain C_{max} (T_{max}) were calculated to be 909.8 ± 1.9 ng/mL and 3 ± 0.0001 h, respectively. $T_{1/2}$ durations were determined to be 5.4 ± 0.02. AUC_{0-t} and $AUC_{0-\infty}$ were calculated to be 6458 ± 15.5 and 6474 15.6 ng h mL^{-1} respectively, using the trapezoidal rule. On the other hand, Ke was determined to have a value of 0.128 ± 0.0005. In addition, C_0, V_d and Cl for ASB sample were observed to be 853 ± 2.9, 386.6 ± 1.3, 50 ± 0.25, respectively. Fig. 6 depicts the representative chromato-

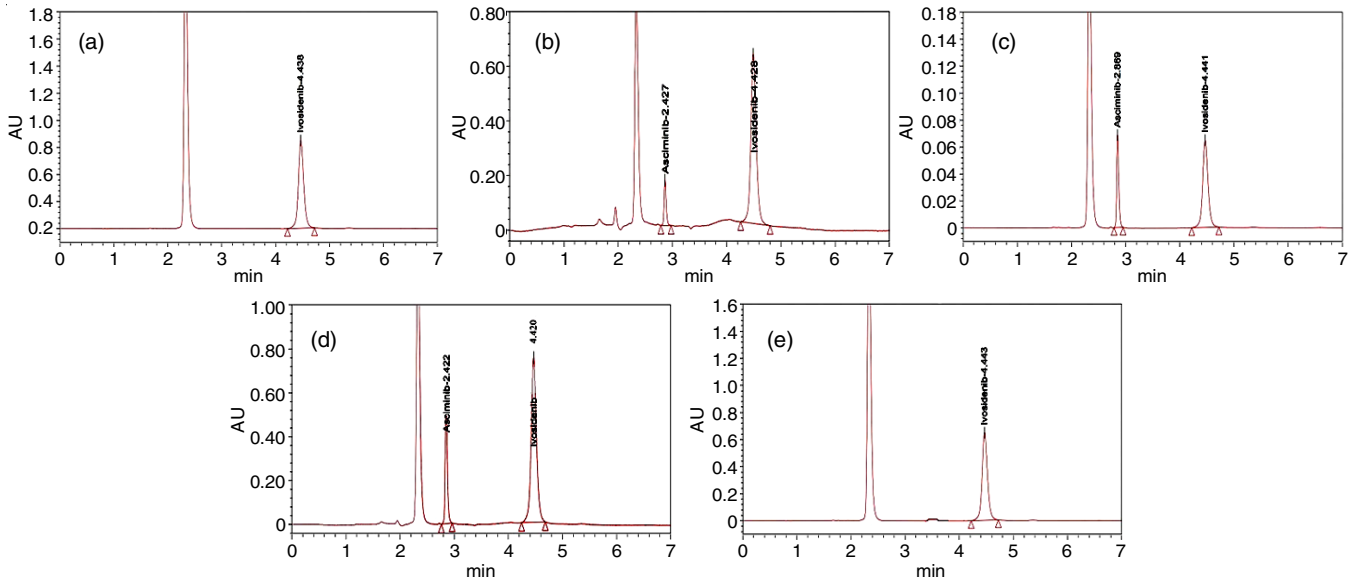

Fig. 6. Demonstration of the effectiveness of drug release of asciminib with representative chromatograms blank plasma at 0 h (a), plasma spiked asciminib collected after 1 h (b), after 3 h (c), after 5 h (d) and 9 h (e)

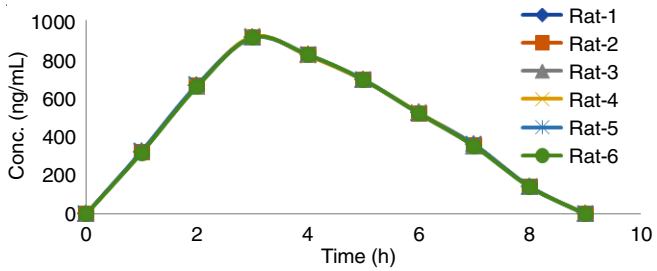

Fig. 5. A bell-shaped curve representing the superposition of plasma concentration-time profiles of asciminib in the plasma of six rats

grams for rat plasma collected at 0, 1, 3, 5 and 9 h after being injected with asciminib. Table-7 displays all the determined pharmacokinetic parameters, which are in excellent agreement with one another, demonstrating the efficient applicability of this method to bioanalytical investigations. Moreover, the method offers sufficient information for preclinical and pharmacokinetic investigations.

TABLE-7
PHARMACOKINETIC PARAMETERS
EXPRESSED AS MEAN ± STANDARD DEVIATION

Parameters	ASB Mean* ± SD
C_{max} (ng mL^{-1})	909.8 ± 1.9
T_{max} (h)	3 ± 0.0001
K_e (h^{-1})	0.128 ± 0.0005
$T_{1/2}$ (h)	5.4 ± 0.02
AUC_{0-t} (ng h mL^{-1})	6458 ± 15.5
$AUC_{0-\infty}$ (ng h mL^{-1})	6474 ± 15.6
C_0 (ng mL^{-1})	853 ± 2.9
V_d (L kg^{-1})	386.6 ± 1.3
Cl (L h^{-1})	50 ± 0.25

*Mean n = 3 determinations

Optimization of the conditions for chromatography: In order to separate and quantify the analyte of interest, a number of different trails were carried out to optimize the chromatographic conditions, which included the mobile phase solvents, composition and ratio of the mobile phase, flow rate and pH of the buffer. All of these factors are collectively crucial. Based on the results of the physico-chemical experiments performed on the target, initial separation attempts were made using a variety of mobile phase solvents in a variety of different combinations. After these failed attempts, a last trial using a composition of phosphate buffer and acetonitrile was able to successfully elute both peaks (asciminib and ivosidenib). The drawback with peak shape, known as tailing, was gradually improved by conducting trials with pH changes. A nice shape was achieved for both medications when the pH was raised to 3 with orthophosphoric acid. The resolution, which was initially low with ratios of 60:40, 50:50 and 30:70, has been improved by altering the ratio of phosphate buffer from acetonitrile to 40:60. The flow rate was maintained at 1 mL per min, which resulted in retention durations for both drugs that were lower than 10 min and 2.8m for asciminib and 4.4 m for ivosidenib.

Conclusion

Bioanalytical HPLC technique was developed and validated for the first time for the determination of asciminib in rat plasma. This procedure is robust, rapid and reproducible and it was verified in accordance with USFDA criteria. On the basis of the data presented in the results and discussions, it can be stated that the developed approach is simple and effective and may be applied in pharmacokinetic investigations to examine the analyte in body fluids.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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