RESEARCH ARTICLE

A Novel Stability Indicating Reversed Phase Ultra Performance Liquid Chromatography Method for Estimation of Asciminib its Bulk and Tablet Formulation

Dandamudi S. Priya[1*], Shivakumar Gubbiyappa[2], Vasanthi Rangapuram[2]

[1]School of Pharmacy, Anurag University, Hyderabad, India
[2]GITAM School of Pharmacy, GITAM Deemed to be University, Hyderabad, India.

Received: 23th October, 2022; Revised: 13th November, 2022; Accepted: 09th December, 2022; Available Online: 25th December, 2022

ABSTRACT

The key objective of the proposed study was to creat and validate an economical, perceptive, specific, reliable and simple reversed phase ultra-performance liquid chromatography (RP-UPLC) method with better response was anticipated for the investigation of asciminib bulk powder and its tablet form. UPLC system (WATERS, Model-2695) connected with PDA (Model-2996) detector was opted to made the present method. To separate asciminib proficiently, method conditions such as C18 column (50 x 4.6 mm, 1.7 μ), mobile system of trifluoro aceticacid (TFA) in water (0.1%) and acetonitrile in 75:25 v/v, 0.3 mL/min flow rate and 260 nm wavelength were used as optimized chromatographic conditions. The anticipated method was validated by the ICH specifications. The retention time of asciminib was noticed at 0.925 minute with good efficiency, respectively**.** Linearity was noticed for concentrations ranging fom 5 to 30 μg/mL of asciminib with R^2 value of 0.999. The %RSD of both system and method precision was assessed in the range of 0.49 to 0.86. The percentage recovery of asciminib was in the range of 100.06–100.6%. The logarithm of the odds (LoD) and limit of quantitation (LoQ) of asciminib determined to be 0.23 and 0.69 μg/mL correspondingly. The results confirmed that the projected technique was economical, simple, responsive and precise. Exploration of asciminib under various FD environments confirms the stability representing the nature of the stated method**.** The proposed UPLC method is extremely valuable in the separation and estimation of asciminib.

Keywords: Asciminib, Sensitivity, Stability indicating, WATERS ultra-performance liquid chromatography, Trifluoro acetic acid.

International Journal of Pharmaceutical Quality Assurance (2022); DOI: 10.25258/ijpqa.13.4.23

How to cite this article: Priya DS, Gubbiyappa S, Rangapuram V. A Novel Stability Indicating Reversed Phase Ultra Performance Liquid Chromatography Method for Estimation of Asciminib its Bulk and Tablet Formulation. International Journal of Pharmaceutical Quality Assurance. 2022;13(4):490-495.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

In contrast to other licensed ABL1 kinase inhibitors, asciminib (ABL001) is a strong, targeted, and orally bioavailable BCR-ABL1 inhibitor that does not bind to the kinase's ATP-binding site.[1-3] Asciminib, in contrast, functions as an allosteric inhibitor and binds to an empty pocket at a location of the kinase domain that the myristoylated N-terminal of ABL1 normally occupies.[2-4] Asciminib was approved recently by FDA in October, 2021[5]. It was marketed has tablet (Scemblix) dosage form by Novarits AG pharma company. Chemically asciminib is N-[4-[chloro(difluoro)methoxy]phenyl]-6-[(3R)-3-hydroxypyrrolidin-1-yl]-5-(1H-pyrazol-5-yl)pyridine-3-carboxamide with molecular formula and molecular weight of $C_{20}H_{18}ClF_2N_5O_3$ and 449.8 g/mol respectively (Figure 1) [6].

As of now, only a single liquid chromatography–mass spectrometry (LC–MS) method has been available for the estimation of the pharmacokinetic properties of the drug in human subjects.[7] Neither a single high-performance liquid chromatography (HPLC) nor ultra performance liquid chromatography (UPLC) method was reported in the literature for asciminib estimation in bulk and tablets. It is very important to have HPLC and UPLC methods in quality control unit of the pharmaceutical industry for routine analysis during the synthesis and formulation of analyte [8]. A stability-indicating

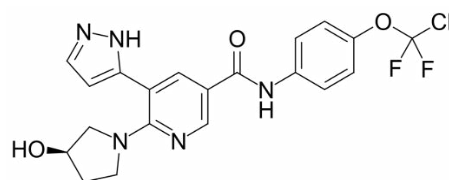


Figure 1: Molecular structures of Ascimini.

**Author for Correspondence: santhoshigitam@gmail.com*

liquid chromatographic approach is essential to determine the stability of the analyte, assessment of % degradation of anlyte and separation of degradants.[9] Hence, research was forwarded to develop a novel stability indicating UPLC method for assessment of asciminib.

MATERIALS AND METHODS

The asciminib pure powder (API) was obtained from ShreeIckon Pharma, Andhrapradesh. All the reagents, and chemicals used in the present investigative study were procured from Fine chemicals, Mumbai.

Chromatographic Conditions

WATERS UPLC system (Model-2695) with PDA detector (Model-2996) was opted to made the present method. To separate asciminib proficiently, method conditions such as C18 column (50x 4.6 mm, 1.7 µ), mobile system of trifluoro acetic acid (TFA) in water (0.1%) and acetonitrile in 75:25 v/v, 0.3 mL/min flow rate and 260 nm wavelength were optimized from trial and error series (Table 1). All the required solutions were prepared by mobile phase as diluents. Ambient conditions were used to protect the column.

Standard Solution Preparation

A 20 mg of asciminib powder was weighed precisely and dissolved to 100 mL with the appropriate diluent to attain a solution of 200 µg/mL of asciminib, which was considered as stock. The 5 mL of the prepared stock solution (200 µg/mL) was promoted to dilution to get 20 µg/mL, which was stated as 100% level concentration.

Sample Solution Preparation

The tablet powder (Scemblix-20 mg) of 94 mg which was corresponding to 20 mg of asciminib was weighed precisely and dissolved to 100 mL with appropriate diluent to attain a solution of 200 µg/mL of asciminib, which was considered as stock. The 5 mL of the prepared stock solution (200 µg/mL) was promoted to dilution to get 20 µg/mL, which was stated as 100% level concentration. Nylon filter (0.25 µm) used to eliminate suspended particles in prepared solution.

Method Validation

The present procedure validation has been ascertained as per ICH (Q2) specifications[10].

System Suitability

It could be confirmed by analyzing asciminib standard solution for six times and recorded chromatograms were examined to assess the % RSD, plate count (N) and peak asymmetry (T).

Linearity

The linearity represented a straight proportional association between input concentrations and output peak areas of the analytical method. In the current method, a series of concentrations ranging from 5 to 30 µg/mL of asciminib was injected, and a liner plot was drawn between input concentrations and output peak areas. Regression coefficient (r^2) and intercept values were computed.

Precision

Precision is the degree to which the results of the homogeneous analyte on various samples are intimately consistent. Usually, it is a function of repeatability and reproducibility. Both repeatability (system precision) and reproducibility (method precision) of the existing method were performed by introducing the solution of standard and sample, respectively in six replications. The % RSD was computed for peak areas and % assay were assessed to confirm the system precision and method precision, respectively.

Accuracy

The anticipated procedure accuracy was done by recovery method, where a definite concentration of the sample has been spiked to various levels of standard solution (50, 100, and 150%). Three replicate injections were introduced to UPLC system at each level of standard level and the mean % recovery of spiked amount of sample was assessed from the resultant chromatograms.

Specificity

The process is demonstrated to be specific when the asciminib is effectively identified by stated method in the presence of supplementary compounds exclusive of any interference. The procedure involved sequentially introducing 0.3 µL of separate distinct solution of the blank, standard, sample, and placebo with standard. The interferences at the RT of asciminib with above stated solutions were present or not interpreted from the chromatograms. To recognize the intrusions from degradants towards asciminib, the investigation was further reinforced by correlating the FD chromatograms of asciminib with the chromatogram of the fresh asciminib standard solution.

Sensitivity

The detection limit (LoD) and quantification limit (LoQ) were assessed by standard deviation (SD) procedure consist of following formulae.

$$LoD = 3.3 \times \sigma/S$$

$$LoQ = 10 \times \sigma/S$$

σ: SD of the intercept

S: Slope of the calibrated linear curve

Robustness

When tiny, deliberate modifications to the method's parameters have no discernible impact on the technique's performance, the method is considered to be robust. In the stated approach slight alterations to flow rate (± 0.1-mL/min) and composition of organic phase (± 1-mL) were made. Asciminib standard solution (20 µg/mL) analyzed with the modified method conditions. The % RSD of obtained responses was determined.

Stability Studies

In these investigations, the drug moiety was purposefully exposed to higher levels of intense stress than accelerated stability settings. These investigations were helpful in determining the dug substance's stability, which is a fundamental factor in creating a stable dosage form.

The forced degradation (FD) investigations were completed in accordance with the ICH's Q1A and QIB requirements [11].

Acid Hydrolysis

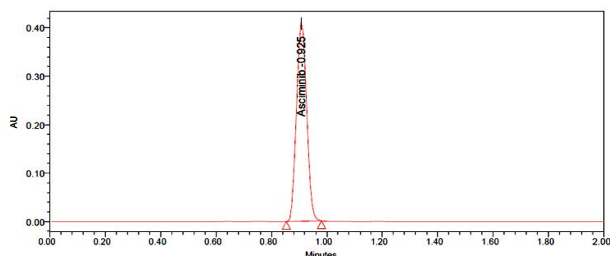
Equal portions of asciminib standard stock solution and 1N HCl solution were combined thoroughly, refluxed at 70°C for 30 minutes, and then set aside to cool. The excess acid was then neutralized by adding 1N NaOH. A solution with 20 µg/mL of asciminib was made by diluting 1-mL of the obtained solution to 50 mL. The final solution was injected for 24 hours with a 6 hour break.

Base Hydrolysis

Equal portions of asciminib standard stock solution and 1N NaoH solution were combined thoroughly, refluxed at 70°C for 30 minutes, and then set aside to cool. The excess acid was then neutralized by adding 1N HCl. A solution with 20 µg/mL of asciminib was made by diluting 1 mL of the obtained solution to 10 mL. The final solution was injected for 24 hours with a 6 hour break.

Oxidative or Peroxide Degradation

Each 10 mL portion of 30% H_2O_2 and asciminib stock solutions were combined thoroughly, refluxed at 70°C for 30 minutes, and then set aside to cool. The excess acid was then neutralized by adding 1N NaOH. A solution with 20 µg/mL of asciminib was made by diluting 1-mL of the obtained solution to 10 mL. The final solution was injected for 24 hours with a 6 hour break.



	Name	RT	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
1	Asciminib	0.925	2459162	100.00		1.06	2620

Figure 2: Chromatogram with optimized conditions.

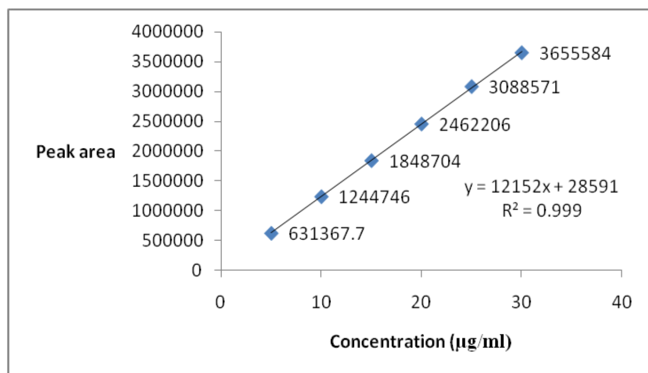


Figure 3: Linearity results of Asciminib

Thermal or Dry heat Degradation

A 10 mL of stock solution of asciminib was placed in heating chamber at 105°C with 75% RH for 24 hours. Further cool the solution and diluted again to get a solution of 20 µg/mL of ssciminib. The final solution was injected for 24 hours with a 6 hour break.

Photo Degradation

A 10 mL of stock solution of asciminib was directly exposed to 254 nm wavelength light in a dark place for 1 day. The stated solution diluted again to attain a solution of 20 µg/mL of asciminib. The final solution was injected for 24 hours with a 6-hour break.

Neutral Degradation

Equal portions of Milli-Q water and stock solution of asciminib were evenly mixed for 30 minutes. The 5 mL of the resultant solution of Asciminib was additionally diluted to 50 mL to acquired a solution of 20 µg/mL of asciminib . The final solution was injected for 24 hours with a 6 hour break.

Assay of Tablets

The % assay of commercial tablets (Scemblix-20 mg) was performed by injecting successive injections of both sample and standard solutions in succession. The % purity of asciminib has been determined by use of peak response thus attained from chromatograms.

RESULTS AND DISCUSSION

Optimized Method

To achieve peaks in the chromatogram with appropriate system sutability, the process was repeatedly tested using various solvents, ratios of mobile systems, and different flow rates (Table 1). Finally, an approach using a C18 column (50 x 4.6 mm; 1.7) with isocratic mode of elution was optimized. The mobile phase used was composed of 0.1% TFA in water and ACN (75:25 V/V). The earlier discussed conditions have been used to validate the approach. With adequate system suitability, these chromatographic conditions can help to elute asciminib at a shorter retention time (RT) of 0.925. (Figure 2).

Method Validation

System suitability parameters includes % RSD, peak asymmetry and column efficiency (plate count) were within the ICH limits (Table 2)

The R^2 value of the asciminib was assessed to be 0.999 for the stated concentration series **(**Figure-3**)** which emphasized that the method has significant linearity for the given range of concentrations. The %recoveries of asciminib in sample at various level of standard solution have been noticed to be 100% ± 2 for asciminib was, indicating the accuracy of the method (Table 3**).** The % RSD of peak areas and % assays of the repeated injections of standard and sample solutions of asciminib was ≤ 2 represented in Table 4. The calculated results appreciably prove the method's precision. The minute and deliberated alterations to method conditions could not influence the methods feat which was assured from the obtained %RSD values (Table 5).

Table 1: Various trials

Trial	*column*	*Flow rate (mL/min)*	*Mobile phase*	*Observation*
1	C18	0.5	ACN: 0.1%OPA (20:80)	Longer RT
2		0.3	0.1%OPA:ACN (40:60)	Peak response is very high
3		0.3	0.1%OPA:ACN (30:70)	Base line was not good
4		0.3	0.1%TFA:ACN (40:60)	Un known peak was observed
5		0.3	0.1%TFA:ACN (80:20)	Broad peak was observed
6		0.3	0.1% TFA: ACN (75:25)	Broad peak was observed

Wave length:242 nm, injection volume: 0.3 µL; trial 6 was considered to be optimized.

Table 2: Results representing the system suitability of standard solution of Asciminib

Injection No.	*Peak area*	*Plate count(N)*	*Tailing factor (T)*
1	2459162	2620	1.06
2	2433584	2619	1.05
3	2447715	2622	1.04
4	2438640	2614	1.05
5	2429551	2629	1.06
6	2428795	2624	1.06
Mean	2439575		
SD	11860.57		
% RSD	0.49		

Table 3: Percentage recovery results Asciminib

%Level	*Amount added (µg/mL)*	*Amount recoverd (µg/mL)*	*%Recovery*	*% Recovery(Mean)*	*Acceptance limit*
50	10	10.1	101.0	101.1	98-102%
	10	10.15	101.5		
	10	10.08	100.8		
100	20	19.8	98.9	100.06	
	20	20.19	101.0		
	20	20.06	100.3		
150	30	30.21	100.7	100.6	
	30	30.27	100.9		
	30	30.06	100.2		

Table 4: Results of precisions of standard solution of Asciminib

Precision	*System precision*	*Method precision*
Mean (n=6)	2439575	99.9
SD	11860.57	0.86
% RSD	0.49	0.86
Acceptance limit	% RSD ≤ 2	

Hence, the method has considerable robustness as of ICH. Interference from blank, degradants and placebo were not seen at RT of asciminib confirming the specificity of the method only towards Asciminib (Figure 4). The LoD and LoQ of asciminib assessed to be 0.23 and 0.69 µg/mL, respectively.

In most stability indicating methods, up to 20% degradation of drug substance or product is considered more effective.[12] The % degradation of asciminib was computed by correlating the peak areas gained from fresh and stressed standard solutions. The peak purity angle and purity threshold of asciminib in various stressed conditions were publicized in Figure 5. The purity threshold value of obtained peaks in chromatograms were higher than purity angle, ensuring the purity of asciminib

Table 5: Results of Robustness of standard solution of Asciminib

Parameter	*Flow rate (0.3 mL/min)*		*Mobile phase (TFA: Acetonitrile 75:25 v/v)*	
	Plus (0.4 mL/min)	*Minus (0.2 mL/min)*	*Plus (74:26 v/v)*	*Minus (76:24 v/v)*
Mean peak area (n=3)	2227305	2864685	2167887	2645851
SD	20119.94	9008.748	21321.82	22176.8
% RSD	0.90	0.31	0.98	0.84

Table 6: %Degradation of Asciminib in various FD conditions

	% Degradation					
Degradation type	*Acid*	*Base*	*Oxidative*	*Thermal*	*Photo*	*Neutral*
Control	0.0	0.0	0.0	0.0	0.0	0.0
0 Hr	6.2	7.7	9.4	2.6	2.1	3
6 Hr	10.6	9.8	10.4	3.1	7.4	3.4
12 Hr	13.3	11.9	14.4	4.2	12.1	4.4
18 Hr	17.6	18.5	20.2	5.3	17.5	5.4
24 Hr	28	28.6	29.6	6.7	20.4	3

Table 7: Assay results of Asciminib Tablets

Drug	*Peak name*	*Peak Area (Mean)*	*%Assay (n=6)*	*SD*	*%RSD*
Asciminib	Standard	2439168	100.1	0.39	0.38
	Test	2441224			

peak with respective to obtained degradants peaks. The assessed % degradation of asciminib results in FD studies significantly shows the stability representative character of the technique (Table 6). The obtained results reveal that asciminib is highly susceptible to acidic, basic and oxidative environments.

Assay of Tablets

The % assay of asciminib tablets was assessed to be 100.1 ± 0.39 9 (Table 7). The obtained results were within the allowable limits (100 ± 2%) as of ICH recommendations.

In common, the stability indicating LC technique is crucial for both qualitative and quantitative drug confirmation.[12,13] Till now, no single HPLC or UPLC method has been available exclusively for the asciminib bulk powder and tablet form. Hence, research has developed a novel UPLC method, which is very important to detect and quantify the very low concentration of analyte in different kinds of samples. In the developed method, a mobile system composition of 0.1% TFA in water and ACN (75:25 V/V) used for a run time of 2 minutes and the retention time of asciminib was observed at 0.92 minutes. These were indicating the methods economical nature and shorter analysis time due to simple solvent systems and shorter elution time. Determination of the degradants and quantification of the % degradation of asciminib in drug substance and tablet formulation by the method significantly ensures the stability representing character of the method. With the current technique, more samples must be assessed quickly. The approach exhibits good reliability, sensitivity, and considerable specificity towards to the testing of asciminib, according to static data of the evaluated parameters.

CONCLUSION

An economical, insightful, specific and simple reverse-phase procedure with better response was established for determination of Asciminib in pure powder and its tablets. Evaluation of asciminib under different forced conditions confirms stability and representative nature of the procedure. The projected technique was competently separate asciminib and possible degradants produced by asciminib under stressed conditions with superior resolution. The anticipated method has shorter retention time for asciminib. Hence, the expected method will get great credit in the pharmaceutical division.

LIST OF ABBREVIATIONS

RSD: Relative Standard Deviation
FD: Forced degradation
SD: Standard Deviation

CONFLICT OF INTEREST

No conflict among the authors and all were agreed to communicate the manuscript to this journal

ACKNOWLEDGEMENT

The authors are greatly thankful to GITAM Deemed to be University, Hyderabad for support and encouragement.

REFERENCES

1. Réa D, Hughes TP. Development of asciminib, a novel allosteric inhibitor of BCR-ABL1. Crit Rev Oncol Hematol. 2022;171:103580.
2. Schoepfer J, Jahnke W, Berellini G, Buonamici S, Cotesta S, Cowan-Jacob SW, Dodd S, Drueckes P, Fabbro D, Gabriel T, Groell JM, Grotzfeld RM, Hassan AQ, Henry C, Iyer V, Jones D, Lombardo F, Loo A, Manley PW, Pellé X, Rummel G, Salem B, Warmuth M, Wylie AA, Zoller T, Marzinzik AL, Furet P. Discovery of Asciminib (ABL001), an Allosteric Inhibitor of the Tyrosine Kinase Activity of BCR-ABL1. J Med Chem. 2018;61(18):8120-8135.
3. Manley PW, Barys L, Cowan-Jacob SW. The specificity of asciminib, a potential treatment for chronic myeloid leukemia, as a myristate-pocket binding ABL inhibitor and analysis of its interactions with mutant forms of BCR-ABL1 kinase. Leuk Res. 2020;98:106458. doi: 10.1016/j.leukres.2020.106458.
4. Yeung DT, Shanmuganathan N, Hughes TP. Asciminib: a new therapeutic option in chronic-phase CML with treatment failure. Blood. 2022 Jun;139(24):3474-3479.
5. Tran P, Hanna I, Eggimann FK, Schoepfer J, Ray T, Zhu B, Wang L, Priess P, Tian X, Hourcade-Potelleret F, Einolf HJ. Disposition of asciminib, a potent BCR-ABL1 tyrosine kinase inhibitor, in healthy male subjects. Xenobiotica. 2020;50(2):160-79.
6. PubChem. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004-. PubChem Compound Summary for CID 72165228, Asciminib; [cited 2022 Sept. 11]

7. Yeung DT, Shanmuganathan N, Hughes TP. Asciminib: a new therapeutic option in chronic-phase CML with treatment failure. Blood, The Journal of the American Society of Hematology. 2022;139(24):3474-9.
8. Chandraman K. An updated review on ultra performance liquid chromatography. Analytical Chemistry: An Indian Journal. 2016;16:1-9.
9. Ngwa G. Forced degradation as an integral part of HPLC stability-indicating method development. Drug delivery technology. 2010;10(5):56-9.
10. Godela R, Gummadi S. A simple stability indicating RP-HPLC-DAD method for concurrent analysis of Tenofovir Disoproxil Fumarate, Doravirine and Lamivudine in pure blend and their combined film coated tablets. InAnnales Pharmaceutiques Françaises 2021;79,(6):640-651.
11. Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs—A review. J Pharm Anal 2014;4:159–65.
12. Hotha KK, Reddy SPK, Raju VK, Ravindranath LK. Forced Degradation Studies: Practical Approach - Overview Of Regulatory Guidance And Literature For The Drug Products And Drug Substances. Int Res J Pharm 2013;4:78-85.
13. Bakshi M., Singh S. Development of validated stability-indicating assay methods—critical review. Journal of Pharmaceutical and Biomedical Analysis. 2002;28(6):1011–1040.