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Research Article

A STABILITY INDICATING RP- HPLC METHOD FOR ESTIMATION OF ASCIMINIB IN API & PHARMACEUTICAL DOSAGE FORM

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ABSTRACT
Asciminib was estimated using RP-HPLC in a simple, precise approach. Stationary phase Agilent C18 (250mm*4.6mm5m), mobile phase 0.01N Kh2po4: Acetonitrile in the ratio 60:40, flow rate 1.0ml/min, detection wave length 224nm, column temperature 30oC, and diluent mobile phase. Optimised conditions were set. System appropriateness characteristics were studied by injecting the standard six times and scoring considerably below acceptability. R2 was 0.999 for linearity study between 25% and 150%.Precision was 0.3 for repeatability and moderate precision.LOD and LOQ are 0.11µg/ml and 0.33µg/ml. The aforesaid approach assayed commercial formulation and found 99.45%. All Asciminib degradation investigations showed purity thresholds greater than purity angle and within acceptable limits. Full length procedure was not conducted; if done, it can be utilised for regular Asciminib analysis.

Key Words: Asciminib, Method development, Validation, RP-HPLC.

INTRODUCTION

Asciminib is a novel, allosteric inhibitor of the BCR-ABL1 tyrosine kinase, specifically targeting the ABL1 myristoyl pocket (STAMP). This mechanism of action distinguishes it from other tyrosine kinase inhibitors (TKIs) used in the treatment of chronic myeloid leukemia (CML), such as imatinib, dasatinib, and nilotinib, which act by competitively inhibiting the ATP-binding site. Asciminib represents a significant advancement in CML therapy, offering an alternative treatment for patients who have developed resistance or intolerance to existing ATP-competitive TKIs.

Chronic myeloid leukemia is a hematologic malignancy characterized by the presence of the Philadelphia chromosome, a genetic abnormality that leads to the production of the BCR-ABL1 fusion protein. This fusion protein exhibits constitutive tyrosine kinase activity, driving the proliferation of leukemic cells. Targeted inhibition of BCR-ABL1 with TKIs has transformed the treatment landscape of CML, offering long-term disease control and improved survival rates. However, resistance due to mutations in the BCR-ABL1 kinase domain or adverse effects from prolonged TKI therapy remains a significant clinical challenge.

Asciminib works by binding to a different site on the BCR-ABL1 protein, the myristoyl pocket, which leads to conformational changes that inactivate the kinase. This unique mechanism not only avoids cross-resistance with ATP-competitive TKIs but also enhances its ability to target certain resistant mutations, such as the T315I mutation, which confers resistance to most other TKIs. Additionally, asciminib has demonstrated efficacy in patients with heavily pre-treated CML and those harboring specific mutations that limit treatment options.

In October 2021, the U.S. Food and Drug Administration (FDA) granted accelerated approval for asciminib under the brand name Scemblix for the treatment of adult patients with Philadelphia chromosome-positive CML

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in chronic phase (CML-CP), either after two or more prior TKIs or for those with the T315I mutation. This approval was based on results from the ASCEMBL trial, which showed asciminib's superiority in achieving major molecular response (MMR) compared to bosutinib in patients who had failed multiple TKI therapies.

Asciminib uniquely targets the ABL1 myristoyl pocket, unlike conventional TKIs that compete for the ATP-binding site. This allosteric inhibition prevents the kinase's active conformation, thereby reducing leukemic cell proliferation. By targeting this alternative site, asciminib can overcome resistance seen with ATP-competitive inhibitors, particularly in cases involving the T315I mutation.[1-10]

Analytical Background[11]

Asciminib exerts its therapeutic activity by inhibiting an oncogenic protein responsible for the proliferation of CML. It may be administered orally once or twice a day depending on the condition being treated. It is chemically known as N-[4-(chlorodifluoromethoxy) phenyl]-6-[(3R)-3-hydroxypyrrolidin-1-yl]-5-(1H-pyrazol-5-yl)pyridine-3-carboxamide

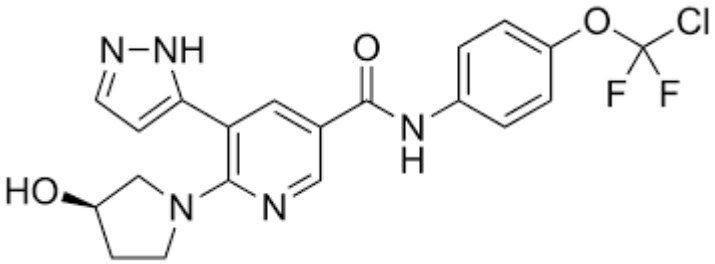

Figure 1 structure of Asciminib

High Performance Liquid Chromatography (HPLC) plays a crucial role in the validation of Asciminib, In the review of literature, more economical methods were observed [12-16], hence a simple, cost-effective stability-indicating simultaneous estimation of Asciminib by RP-HPLC in pharmaceutical dosage form must be developed and validated as per the guidelines of ICH (Q2 specification).

MATERIALS

Asciminib pure drug (API), Asciminib formulation (Scemblix), Distilled water,Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

INSTRUMENTATION

The development and method validation were conducted using a WATERS HPLC, specifically the model 2695 SYSTEM, equipped with a Photo diode array detector. The system also included an automated sample injector and the Empower 2 software.

Table 1: Chromatographic Conditions

Mobile phase	**0.01N Kh2po4: Acetonitrile (70:30 v/v)**
Flow rate	1.0 ml/min
Column	Agilent C18 (4.6 x 150mm, 5µm)
wave length	224 nm
Column temperature	30°C
Injection volume	10µL
Run time	10.0 min
Buffer	0.01N Kh2po4

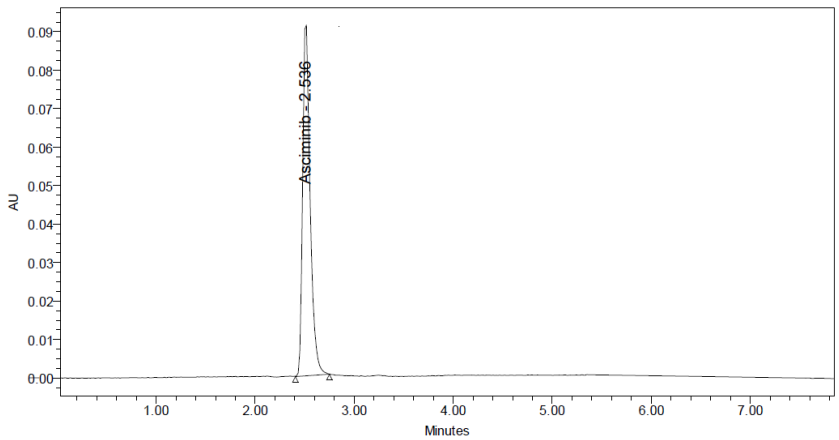

Figure 2: Optimized Chromatogram

Methods:

Preparation of Standard stock solutions: Accurately weighed 10mg of Asciminib transferred 50ml and volumetric flasks, 3/4 The of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (200µg/ml of Asciminib).

Preparation of Standard working solutions (100% solution): 1ml of Asciminib from stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (20µg/ml of Asciminib).

Preparation of Sample stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (200 µg/ml of Asciminib).

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (20µg/ml of Asciminib)

Validation:

System suitability parameters:

The system suitability parameters were determined by preparing standard solution of Asciminib (20 ppm) and the solution were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be more than 2%.

Specificity (Selectivity): Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific. Representative chromatogram is shown in Figure 3 and experimental data is given in Table 2.

Table: 2 System suitability parameters for Asciminib

S no	Asciminib		
Inj	RT(min)	USP Plate Count	Tailing
1	2.521	6023	1.60
2	2.528	6069	1.59
3	2.530	5912	1.60
4	2.535	6089	1.59
5	2.540	6219	1.59
6	2.543	6328	1.58

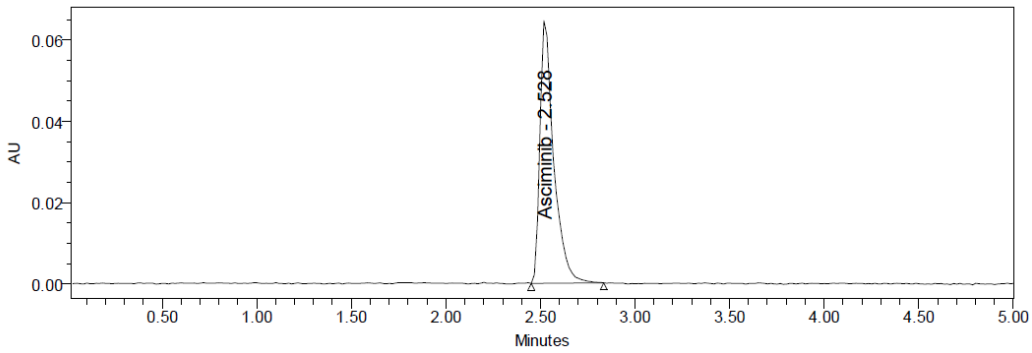

Figure 3: System Suitability Chromatogram of Asciminib

Table 3: Specificity Data

Peak name	Rt	Area	USP plate count	Tailing
Asciminib	2.536	4897323	6191.3	1.3

Specificity:

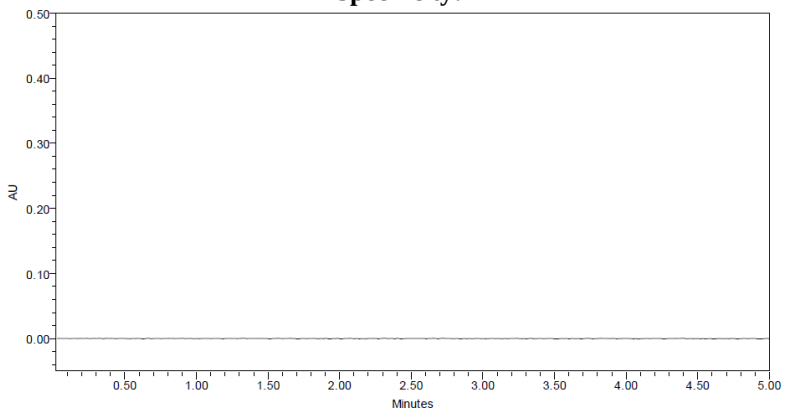

Figure 4 Chromatogram of blank.

The forced degradation conditions are mentioned in Table 4 and the results are mentioned in Table 5.

Table 4: Forced degradation conditions for Asciminib

Stress condition	Solvent	Temp(^{0}C)	Exposed time
Acid	2N HCL	60^{0}c	30 mins
Base	2N NAOH	60^{0}c	30 mins
Oxdation	20% H_2O_2	60^{0}c	30 mins
Thermal	Diluent	105^{0}c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^{0}c	

From the results, degradation peaks were observed when the samples were exposed to acid. According to the stress study, none of the degradant co-eluted with the active drug peaks formed.

Table 5: Degradation profile results

Degradation Conditon	% Drug UnDegeraded	% Drug Degeraded
Acid	94.69	5.31
Base	95.80	4.20
Oxidation	95.50	4.50
Thermal	97.91	2.09
Photolytic	97.77	2.23
Hydrolytic	99.26	0.74

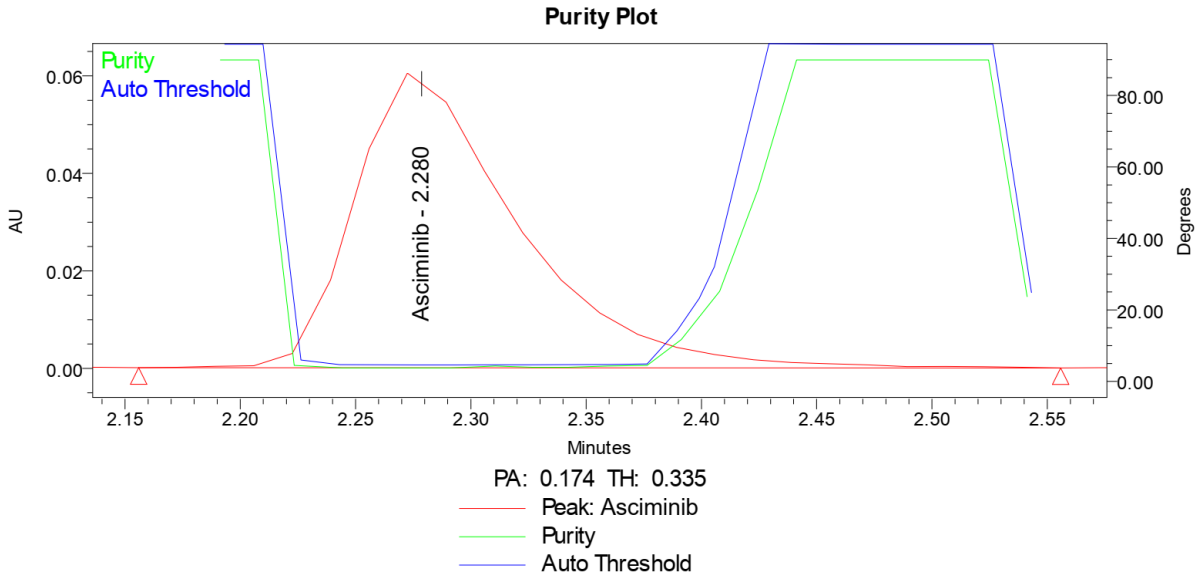

Figure 5: Purity Plot of Acid

Limit of detection (LOD) The detection limit is considered as very low level of concentration of an analyte in a sample that can be detected, but not necessarily quantitated.

Limit of quantitation (LOQ): The limit of quantitation is considered as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy of the method.

The LOD values obtained for Asciminib are listed in Table 6.

Table 6: Summary of limit of detection

Sample	Conc (µg/ml)
LOD	0.11
LOQ	0.33

Linearity: The linearity of the method was demonstrated for Asciminib by analyzing the solutions ranging from 25% to 150% of the specification limit (Table 7). The correlation coefficient for Asciminib was 0.999. This indicates good linearity

Linearity:

Calibration data is given in table 7 and regression data in table 8 and calibration curve in figure 6

Table 7: Calibration data of Asciminib

Asciminib	
Conc (µg/mL)	**Peak area**
0	0
5	121620
10	245371
15	363179
20	487249
25	606587
30	722803

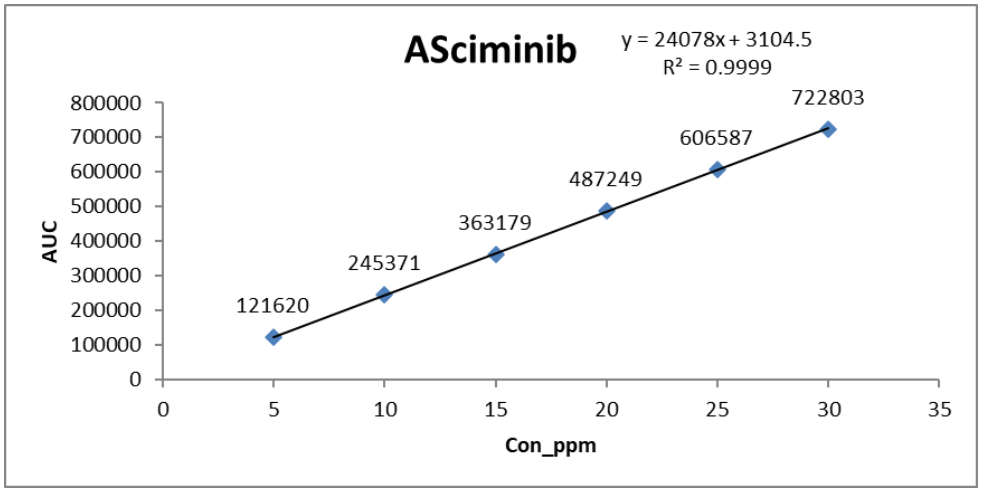

Figure 6: Calibration curve of Asciminib

Table 8: regression data

Parameter	Asciminib
Conc range (µg/mL)	5-30µg/ml
Regression Equation	y = 24078x + 3104.5
Co-relation	0.999

Accuracy: The accuracy of the method was determined by using solutions containing spiked samples of Asciminib at 50%, 100% and 150% of the working strength. All the solutions were prepared in triplicate and analysed. The percentage recovery results obtained for each impurity was listed in Table 9

Table 9 Accuracy table of Asciminib

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% recovery
50%	10	9.93	99.32
	10	9.99	99.93
	10	9.82	98.17
100%	20	19.83	99.13
	20	19.99	99.97
	20	19.99	99.94
150%	30	29.86	99.53
	30	29.74	99.12
	30	29.99	99.95
Mean % recovery			**99.45**

System Precision: The system precision was performed by analyzing six replicate injections of standard solution at 100% of the specified limit with respect to the working strength of Asciminib. Results of peak area are summarized in Table 10.

Table 10 System precision table of Asciminib

S. No	Area of Asciminib
1.	485755
2.	488874
3.	486875
4.	485754
5.	484174
6.	485847
Mean	**486213**
S.D	**1564.0**
%RSD	**0.3**

Method Precision: The precision of the method was determined by analyzing a sample of Asciminib). Data obtained is summarized in Table 11

Table 11 Repeatability table of Asciminib

S. No	Area of Asciminib
1.	486390
2.	483430
3.	486238
4.	486698
5.	483767
6.	485574
Mean	**485350**
S.D	**1409.2**
%RSD	**0.3**

Intermediate precision: It is differently from the repeatability, the precision obtained within a single laboratory over a longer period (generally at least several months) and considers more changes than repeatability. Data obtained is summarized in Table 12

Table 12 Intermediate precision table of Asciminib

S. No	Area of Asciminib
1.	481846
2.	481139
3.	481249
4.	482897
5.	481532
6.	481855
Mean	**296367**
S.D	**633.7**
%RSD	**0.2**

Robustness: The chromatographic conditions were deliberately changed to evaluate the robustness of the existing method. To determine the robustness of method, system suitability solution is prepared as per methodology and injected into HPLC at different altered conditions to check the method's ability like flow rate (± 10%), column oven temperature (± 5°C) and Mobile phase (± 10%) from actual method conditions. No significant change is observed by changing flow, temperature, Mobile phase, and system suitability also complied as per methodology. The robustness results are summarized in Table 13.

Table 13 Robustness data for Asciminib

Condition	%RSD of Asciminib
Flow rate (-) 0.9ml/min	0.5
Flow rate (+) 1.1ml/min	0.7
Mobile phase (-) 55B:45A	0.2
Mobile phase (+) 65B325A	0.1
Temperature (-) 27°C	0.4
Temperature (+) 33°C	0.2

Assay data:- Scemblix Tablet bearing the label claims Asciminib 300 mg. Assay was performed with the above formulation. Average % Assay for Asciminib obtained was 99.62%. Assay data shown in table no 14.

Figure 7: Asciminib Marketed Drug

Formula to calculate assay:

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{1}{10} \times \frac{10}{1} \times \frac{P}{5} \times \frac{FV}{100} \times \frac{}{L.C} \times 100$$

AT	Avergage peak area of sample in test solution
AS	Mean peak area of sample in standard solution
WS	Weight of sa,ple working standard taken in mg
P	Assay of sample working standard in % in dried basis
L.C	Label claim
FV	filled volume (1ml of a vail)

Table 14: Assay Data of Asciminib

S.no	Standard Area	Sample area	% Assay
1	485755	486390	99.84
2	488874	483430	99.23
3	486875	486238	99.81
4	485754	486698	99.90
5	484174	483767	99.30
6	485847	485574	99.67
Avg	**486213**	**485350**	**99.62**
Stdev	**1564.0**	**1409.2**	**0.29**
%RSD	**0.3**	**0.3**	**0.3**

CONCLUSION

The Asciminib HPLC investigation found that this approach can properly measure the drug's concentration and purity. This technology is ideal for pharmacokinetic studies and standard quality control since it may be used repeatedly with exact peak resolutions and steady retention times. HPLC analysis is used to determine Asciminib's efficacy and safety in medicinal applications, as well as to verify its chemical makeup.

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