NOVEL METHOD DEVELOPMENT AND VALIDATION OF FORCED DEDRADATION STUDY FOR THE ASSAY OF TYROSINE KINASE INHIBITOR IN ASSAY FOR PHARMACEUTICAL DOSAGE FORM BY RP - UPLC

Badikela Rama Krishna,[1*] Shiva Prasad Kasarapu,[2] V.Haritha,[3] V.Sai Meghana,[4] Shaik Harun Rasheed.[5]

[1*].Department of Pharmaceutical analysis and Quality Assurance, at Guru Nanak Institutions Technical Campus - School of Pharmacy (Autonomous), Ibrahimpatnam, Ranga Reddy - 501506, Telangana, India.

[2].Department of Pharmaceutical sciences, at University of Greenwich Medway, Kent United Kingdom-ME4 4TB.

[3].Department of Pharmacy Practices, at Guru Nanak Institutions Technical Campus - School of Pharmacy (Autonomous), Ibrahimpatnam, Ranga Reddy - 501506, Telangana, India.

[4.5].Department of Pharmaceutics, at Guru Nanak Institutions Technical Campus - School of Pharmacy (Autonomous), Ibrahimpatnam, Ranga Reddy - 501506, Telangana, India.

*Correspondence Author *

Dr. Badikela Rama Krishna.[1]* Associate Professor, Department of Pharmaceutical Analysis and Quality Assurance, at Guru Nanak Institutions Technical Campus - School of Pharmacy (Autonomous), Ibrahimpatnam, Ranga Reddy - 501506, Telangana, India.

ABSTRACT:

The primary aim of this study was to create and validate a straightforward, precise, sensitive, and accurate RP-UPLC method for quantifying asciminib in both pure and dosage forms. The UPLC method was established utilizing the Waters Acquity liquid chromatographic system along with a C18 (150x1.5mm, 1.7µm) column. Validation of the developed method was conducted in accordance with the guidelines set forth by the International Conference on Harmonization (ICH). Effective chromatographic separation of asciminib with satisfactory resolution was accomplished using a mobile phase composed of acetonitrile and a 0.1% formic acid buffer (supplemented with ammonium formate) in a 30:70 v/v ratio, at a flow rate of 0.3 mL/min, an injection volume of 5 µL, and a wavelength of 243 nm. The validated method demonstrated linearity within the concentration range of 5 - 50 µg/mL. The limit of detection (LOD) and limit of quantification (LOQ) for asciminib were determined to be 0.3 and 0.11 µg/mL, respectively. The percentage relative standard deviation (% RSD) was recorded at less than 2%, indicating the precision of the method developed. Furthermore, the recovery rate was found to be nearly 100%, which confirms the accuracy of the method. Minor modifications in the chromatographic conditions indicated the robustness and ruggedness of the method developed. The analytical method established is simple, precise, sensitive, and reproducible, making it suitable for the determination of asciminib.

Keywords: Asciminib, UV, RP-UPLC, Validation, Forced degradation study and ICH Guidelines.

1. INTRODUCTION:

In comparison to other licensed ABL1 kinase inhibitors, asciminib (ABL001) is a potent, targeted, and orally bioavailable BCR-ABL1 inhibitor that does not interact with the ATP-binding site of the kinase. Unlike other inhibitors, asciminib acts as an allosteric inhibitor, binding to an unoccupied pocket in the kinase domain that is typically occupied by the myristoylated N-terminal of ABL1.[1]. Recently, in October 2021, asciminib received approval from the FDA. It is marketed in tablet form (Scemblix) by Glenmark Pharmaceuticals. Chemically, asciminib is identified as N-[4-[chloro(difluoro)methoxy]phenyl]-6[(3R)3hydroxypyrrolidin1yl]5(1Hpyrazol5yl)pyridine-3-carboxamide, with a molecular formula of $C_{20}H_{18}ClF_2N_5O_3$ and a molecular weight of 449.8 g/mol (Fig.1). Currently, there is only two liquid chromatography–mass spectrometry (LC–MS) method available for estimating the pharmacokinetic properties of this drug in human subjects.[2]. one high-performance liquid chromatography (HPLC) or ultra-performance liquid chromatography (UPLC) methods have been documented in the literature for the estimation of asciminib in bulk or tablet forms, but few dropback are observed. The availability of HPLC and UPLC methods is crucial for the quality control units within the pharmaceutical industry, facilitating routine analysis during the synthesis and formulation of the analyte. A stability-indicating liquid chromatographic method is vital for assessing the stability of the analyte, determining the percentage degradation of the analyte, and separating its degradants. Therefore, research efforts have been directed towards developing a novel stability-indicating UPLC method for the assessment of asciminib.[3].

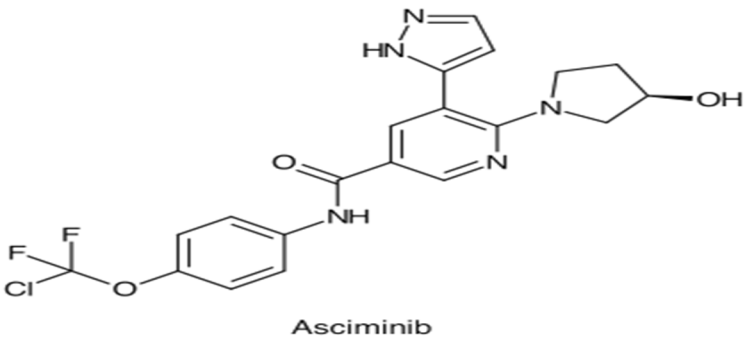

Fig.1: Chemical Structure of Asciminib.

2. EXPERIMENTAL:

2.1. Reagents and Chemicals

Acetonitrile and Formic acid buffer with 0.1% ammonium formate added were utilized, together with Asciminib (Fig. 1) pure bulk drug (API) and Asciminib dosage form tablets (Scemblix). All of the solvents of HPLC quality were supplied by Merck. Every solution and solvent used was filtered using a Whatsman filter (0.25 µ). Glenmark Pharmaceuticals in Mumbai, India, provided the asciminib medication sample as a gift.[4].

2.2. Instrumentation and Conditions for Chromatography

Weighing the compounds was done using an electronic balance. Sis-co businesses used a pH meter to measure the buffers' pH. We used an ultrasonicator-probe to sonicate or degas the solutions. A PDA detector with empower 2 software was used to detect the signal after the chromatography analysis was completed using a Waters Acquity liquid chromatographic machine. In order to design and validate the UPLC technique, a C18 (2.1 x 50 x 1.7 µm),1/PK column was used. The mobile phase consisted of acetonitrile: Formic acid buffer with 0.1% ammonium formate (30:70 v/v) injected at a flow rate of 0.3 mL/min. Five microliters of the sample were injected. The temperature in the column was ambient. The wavelength of 243 nm was selected from the asciminib UV spectrum because it exhibits the highest absorption.[5].

3.MATERIALS AND METHODS:

3.1.Chemicals and Reagents

Asciminib pure bulk medicine (API), asciminib dosage form tablets (Scemblix), acetonitrile, ortho-phosphoric acid, and ammonium dihydrogen phosphate, as well as formic acid buffer containing 0.1% ammonium formate, were employed. Glenmark Pharmaceuticals, located in Mumbai, India, provided the API. All HPLC-grade solvents were provided by Merck, Mumbai, India. All solvents and solutions were filtered using a 0.22 µ Whatsman filter.[6].

3.2. Instrumentation and Conditions for Chromatography

The chemicals were measured using an electronic balance from Infra Digi. The pH levels of the buffers were assessed with a pH meter from RI. The analytical weighing balance exhibited a sensitivity range of 0.1 to 0.001 mg. The pH meter demonstrated a sensitivity of ±0.1. The solutions underwent sonication or degassing with an ultrasonicator from Sisco Enterprises. Chromatographic analysis was performed utilizing the Waters Acquity liquid chromatographic system, with signal detection achieved through a PDA detector in conjunction with Empower 2 software. Method development and validation were executed

on a C18 column (2.1 mm × 50 mm, 1.7 µm)1/pk, employing a mobile phase composed of acetonitrile and formic acid buffer (supplemented with ammonium formate) in a 30:70 v/v ratio, at a flow rate of 0.3 mL/min. The volume of the sample injection was 5 µL. The column was maintained at ambient temperature. Based on the UV spectrum of asciminib, a wavelength of 243 nm was selected, which exhibits the highest absorbance.

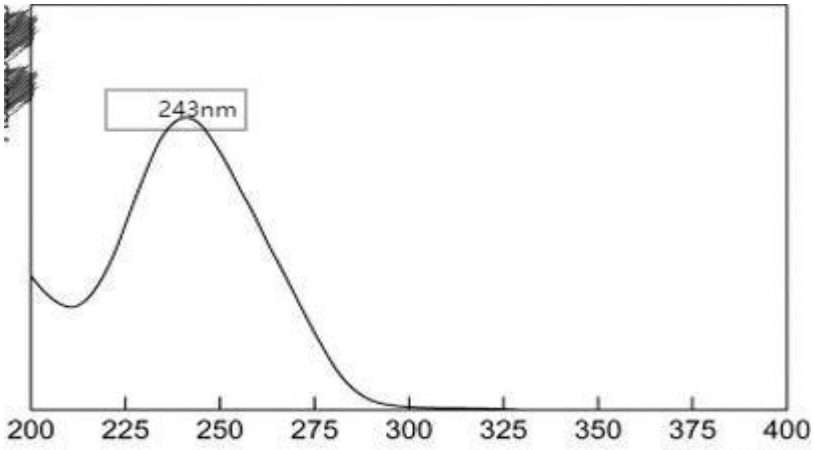

Fig.2: Wavelength of Asciminib

3.3. Preparation of Solutions

i).Mobile Phase

The mobile phase employed for elution consisted of acetonitrile and formic acid buffer (supplemented with ammonium formate) in a 30:70 v/v ratio. A 0.22 µm filter was utilized to filter the mobile phase. The choice of diluent was determined by the solubility of the drugs. DMSO was chosen as the diluent.

ii). Preparation of Standard Stock Solution

A precise measurement of 40 mg of asciminib was placed into a 100 mL volumetric flask. Subsequently, 65% of the diluents were added to the flask. The flask was then subjected to sonication for a duration of 15 minutes. Finally, the flask was filled to the mark with diluent.

iii). Preparation of Standard Working Solution

A standard stock solution of 5 mL was moved to a 50 mL volumetric flask. 65% of the diluents were added to the flask. Subsequently, the diluent was utilized to adjust the volume.

iv).Preparation of Sample Stock Solution

The mean weight of ten pills was assessed. After the tablets were ground into a powder, the weight equivalent to one pill was measured. This powder was subsequently transferred to a volumetric flask with a capacity of one hundred millilitres. Diluents were added to achieve a total volume of 100 mL, which was then filtered through a 0.45 µm syringe filter.

v).Preparation of Sample Working Solution

Filtrated sample stock solution of five millilitres was moved into a volumetric flask with a capacity of 50 mL. Then, the solution was prepared with made up with diluent.

4. ASSAY OF ASCIMINIB:

A total of 16 pills were weighed and ground into a powder. A volumetric flask with a capacity of 100 mL was filled with powder equivalent to 40 mg of asciminib. 65 mL of diluents were added, the mixture was dissolved through sonication, diluted to the desired volume using diluents, and subsequently filtered using a membrane filter with a porosity of 0.45 µm.

5.RESULTS AND DISCUSSION:

5.1.Chromatographic Optimization

Following a series of experiments, the composition of the mobile phase was refined, yet the system suitability parameters did not meet the acceptance criteria. The mobile phase utilized consisted of acetonitrile and formic acid buffer (supplemented with 0.1% ammonium formate) in a 30:70 v/v ratio. The stationary phase employed was C18 (2.1mm × 50mm, 1.7µm)1/pk, operating at a flow rate of 0.5 mL/min, which demonstrated peaks with a favorable theoretical plate count and tailing factor. Consequently, this method was optimized and validated. The Waters Acquity UPLC auto sampler facilitated the elution, method development, and validation of asciminib. The optimized chromatogram is presented in Figure 3.

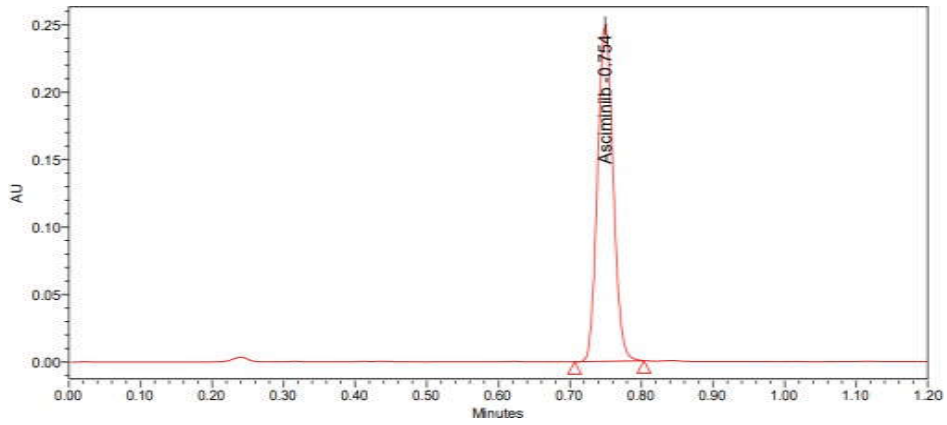

Fig.3: Optimized Chromatogram trial of asciminib

5.2.System Suitability Parameters

The analytical processes involved testing for system compatibility. In accordance with the ICH guidelines, a system suitability test was commonly employed to evaluate the resolution, column efficiency, and repeatability of a chromatographic system, ensuring its appropriateness for a specific analysis. The new methodology was optimized to yield a symmetrical peak and a high number of theoretical plates. The total count of theoretical

plates exceeded 2000, which was considered adequate for the system suitability test. As per the established standards, the tailing factor fell within the prescribed limits. These results indicate that the proposed method can generate data of satisfactory quality. The findings were presented in Table 1.

Table.1: System suitability parameters of asciminib

S. No	RT (min)	USP plate count	Tailing
1	0.765	5632	1.03
2	0.756	5623	1.12
3	0.762	5590	1.11
4	0.751	5600	1.12
5	0.765	5558	1.06
6	0.759	5657	1.05
Mean	0.766	5694	1.10

5.3.Linearity

The linearity of the analytical method was defined by its ability to yield test results within a specific range that were directly proportional to the concentration of the analyte present in the test sample. The regression line analysis illustrates the correlation between the concentration and the peak area of asciminib. Consequently, the results indicated a strong relationship between the peak area and the concentration of the analyte. The high R^2 value demonstrated excellent linearity. The linearity of the analytical method was assessed using six concentration levels ranging from 5 to 50 µg/mL. The regression coefficient, y-intercept, and slope of the regression line were computed. The observed correlation coefficient was found to be 0.995. The results are presented in Table.2

Table.2: Linearity study of Asciminib

S. No	Concentration (µg/mL)	Peak area
1	5.00	326971
2	10.00	731459
3	20.00	1357935
4	30.00	2134756
5	40.00	2619390
6	50.00	3193458

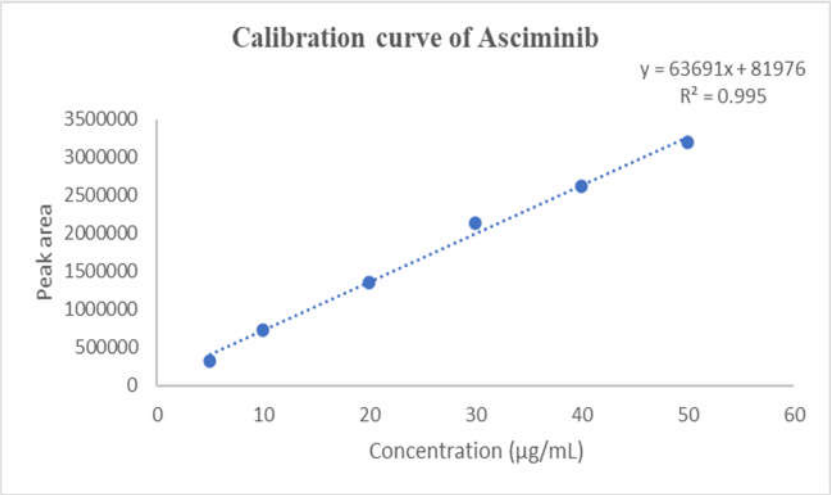

Fig. 4: Calibration plot of asciminib

5.4. Accuracy

The accuracy results demonstrated a percentage recovery across all three levels ranging from 99.90% to 101.30%, with a % RSD of 0.16%, as illustrated in Table 3. The percentage recovery and % RSD values were within the acceptable limits of 98.0% to 102.0% and not exceeding 2.0%, respectively, indicating the method's appropriateness for conducting repetitive drug analyses.

Table.3: Recovery study of asciminib

Recovery level	Amount spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % recovery
50%	20.00	19.93	99.90	100.50±0.8
	20.00	20.26	101.30	
	20.00	20.09	100.50	
100%	40.00	40.09	100.20	100.30±0.36
	40.00	40.00	100.00	
	40.00	40.26	100.70	
150%	60.00	60.27	100.50	100.67±0.29
	60.00	60.27	100.50	
	60.00	60.57	101.00	
Mean				100.39±0.19
SD				0.19
RSD %				0.16

5.5.Precision

The level of concordance among a series of measurements taken from repeated samples of the same uniform material under defined conditions was regarded as the precision of the method, which is generally expressed as relative standard deviation. The % RSD value for the method precision results of asciminib was recorded at 0.39%. Meanwhile, the % RSD value for the intermediate precision results of asciminib was noted at 0.65%. These results were significantly below the commonly accepted threshold of 2 percent. Consequently, the precision of the new method has been validated. The findings were presented in Tables 4 and 5.

5.6.Sensitivity

The limit of quantification (LOQ) is defined as the smallest quantity of analyte in a sample that can be quantitatively identified with suitable accuracy. Conversely, the limit of detection (LOD) is defined as the minimum amount of analyte in a sample that can be detected, although it may not be quantitated. For asciminib, the LOD and LOQ were determined to be 0.12 and 0.4 µg/mL, respectively.

Table. 4: Method precision study of asciminib

S. No	Area of asciminib
1	2622418
2	2611582
3	2614570
4	2628269
5	2615321
6	2637651
Mean	2621635±9919.56
SD	9919.46
RSD%	0.39

5.7.Specificity and Selectivity

The specificity and selectivity of the method were evaluated by examining interference peaks in the chromatograms of blank and placebo samples. Within the retention time ranges, the UPLC chromatograms for the drug matrix (a combination of the medication and placebos) showed almost no interference peaks. Therefore, the method proposed in this study was deemed selective.

Table.5: Intermediate precision study of asciminib

S. No	Area of asciminib
1	2653214
2	2613206
3	2615427
4	2626539
5	2600362
6	2641488
Mean	2625039±19534.66
SD	19534.65
RSD%	0.66

5.8. Robustness

The impact of minor modifications in chromatographic parameters was utilized to assess the reliability of the analytical procedure. The percent RSD of the asciminib assay remained below 2.0 across all intentionally altered chromatographic conditions. The system suitability criteria remained unchanged during the variation of conditions, thereby confirming the robustness of the technique. The results are presented in Table 7.

5.9.Assay

The primary characteristic of the proposed method is its application in the analysis of formulations. Consequently, a market sample of asciminib (Scemblix) was collected and analyzed using the suggested method. As per the label claim, the drug content derived from the sample solution values was determined to be within the acceptable range of 98 - 110 percent. The assay percentage of asciminib was recorded at 100.3±0.3 %. The research demonstrated that the developed UPLC technique is both accurate and user-friendly for daily use. The results are presented in Table 7.

Table.6: Robustness study of asciminib

Condition	RT (min)	Tailing factor	% RSD
Flow rate (-) 0.45 mL/min	0.925	1.12	0.62
Flow rate (+) 0.55 mL/min	0.632	1.11	0.21
Mobile phase (-) 18:82	0.903	1.14	0.21
Mobile phase (+) 22:78	0.625	1.10	0.21

Table.7: Marketed formulation analysis of asciminib

Brand	Label claim (mg)	Amount found (mg)	% Assay
Scemblix	40.00	40.32±0.16	100.3±0.3 %

6.FORCED DEGRADATION STUDIES:

Asciminib was subjected to a variety of stress conditions, including hydrolysis, base, acid, photolytic, reductive, and thermal degradation, in accordance with ICH guidelines. The proposed UPLC method was employed to consistently monitor the degradation behavior. The results from the PDA detector during the forced degradation indicated that the asciminib peaks remained pure and homogeneous across all the stress conditions examined. The purity angle was below the threshold in all degradation scenarios. Furthermore, the drug exhibited greater degradation under peroxide conditions and lesser degradation under hydrolysis conditions. The significant degradation observed in peroxide conditions may be attributed to oxidation-sensitive groups (such as aromatic heterocycles). Conversely, the minimal degradation in hydrolytic conditions suggests that the drug does not possess easily hydrolyzable bonds. Degradant peaks were observed in acid, thermal, and base conditions. The degradation products resulting from acid conditions may stem from amide-N hydrolysis and modifications of heterocycles. The products from base degradation could be a result of nucleophilic attacks and amide cleavage. Elevated temperatures during thermal degradation may have led to bond cleavage or rearrangements. All findings from the stability studies were presented in below Tables, while the chromatogram peaks from the degradation studies were illustrated in Figures:4-9.

Table.8: Acid degradation study of asciminib

Time	Peak area	% Recovered	% Degraded	Tailing factor
0hr	2624786	96.65	3.92	1.04
6 hrs	2556301	90.36	10.13	1.02
12 hrs	2336589	86.66	15.59	1.00
18 hrs	2398547	80.65	19.53	1.09
24 hrs	2998959	76.56	23.63	1.05

Table.9: Alkali degradation study of asciminib

Time	Peak area	% Recovered	% Degraded	Tailing factor
0:0hr	2638564	96.99	3.26	1.07
6 hrs	2394154	91.19	9.01	1.03
12 hrs	2393052	86.56	13.65	1.08
18 hrs	2158547	82.09	18.66	1.07
24 hrs	2069953	78.91	21.01	1.02

Table.10: Reduction degradation study of asciminib

Time	Peak area	% Recovered	% Degraded	Tailing factor
0:0hr	2698594	97.31	2.71	1.10
6 hrs	2466845	91.95	8.12	1.16
12 hrs	2279958	87.02	13.01	1.03
18 hrs	2176478	83.15	16.93	1.06
24 hrs	2120963	81.02	19.01	1.02

Table.11: Thermal degradation study of asciminib

Time	Peak area	% Recovered	% Degraded	Tailing factor
0:0hr	2667854	98.03	2.01	1.10
6 hrs	2300310	95.54	4.53	1.06
12 hrs	2360625	90.13	9.92	1.03
18 hrs	2295968	87.33	12.76	1.01
24 hrs	2245673	85.72	14.31	1.00

Table.12: Photolytic degradation study of asciminib

Time	Peak area	% Recovered	% Degraded	Tailing factor
0:0hr	2579578	98.25	1.86	1.02
6 hrs	2565587	97.91	2.11	1.00
12 hrs	2636214	96.82	3.21	1.11
18 hrs	2398896	95.32	4.71	1.16
24 hrs	2545615	93.39	6.61	1.15

Table.13: Hydrolyze degradation study of asciminib

Time	Peak area	% Recovered	% Degraded	Tailing factor
0:0hr	2389646	98.73	1.32	1.05
6 hrs	2553756	97.59	2.38	1.00
12 hrs	2542021	97.00	2.89	1.07
18 hrs	2621553	96.31	3.72	1.13
24 hrs	2605431	95.72	4.31	1.17

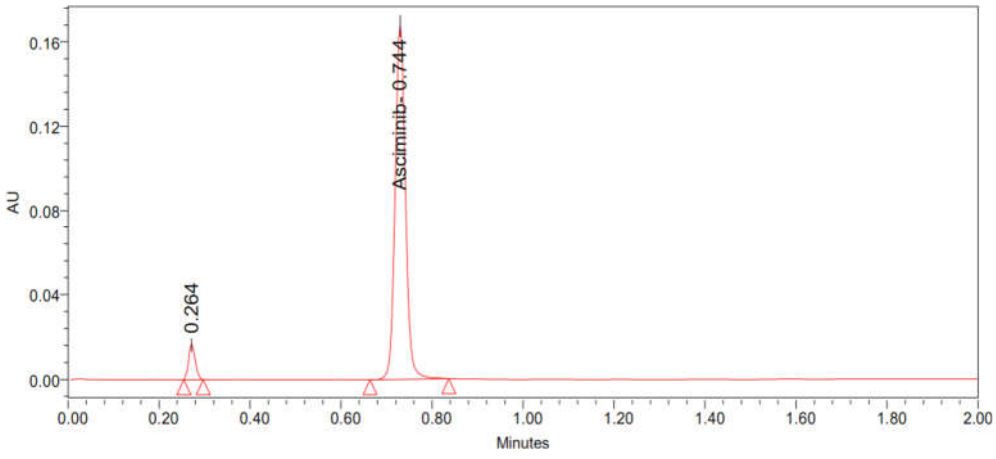

Fig.5: Acid degradation chromatogram of asciminib

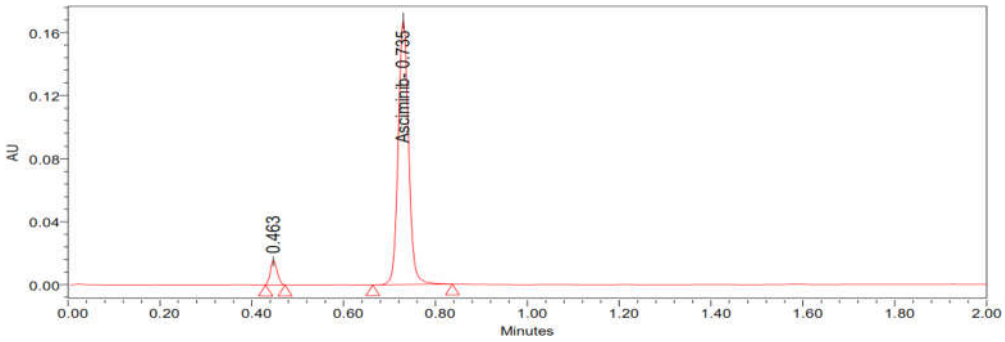

Fig.6: Alkali degradation chromatogram of asciminib

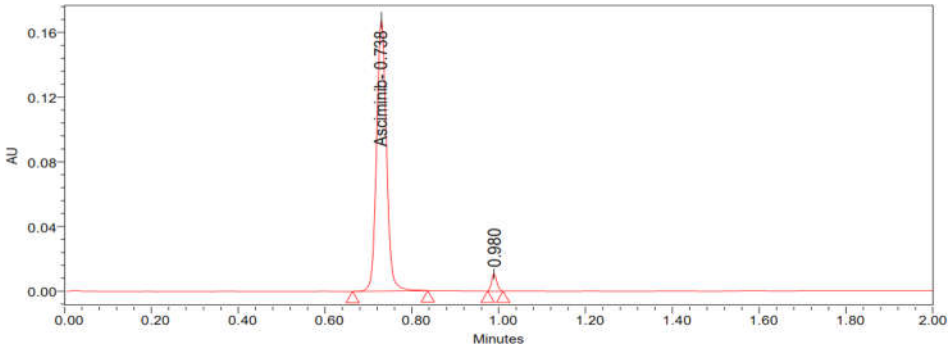

Fig.7: Reduction degradation chromatogram of asciminib

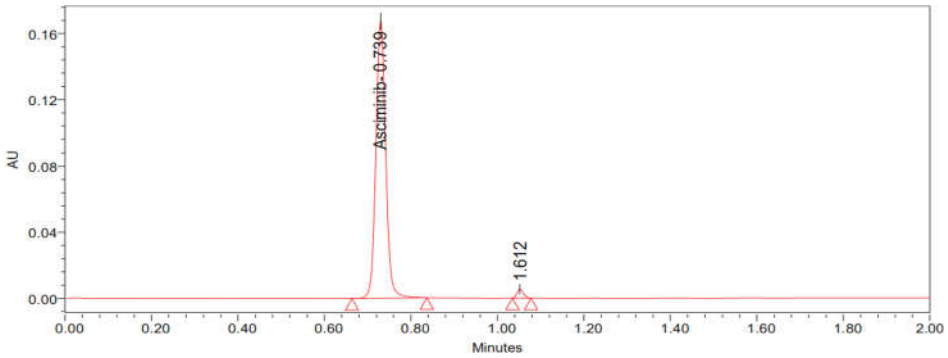

Fig.8: Thermal degradation chromatogram of asciminib

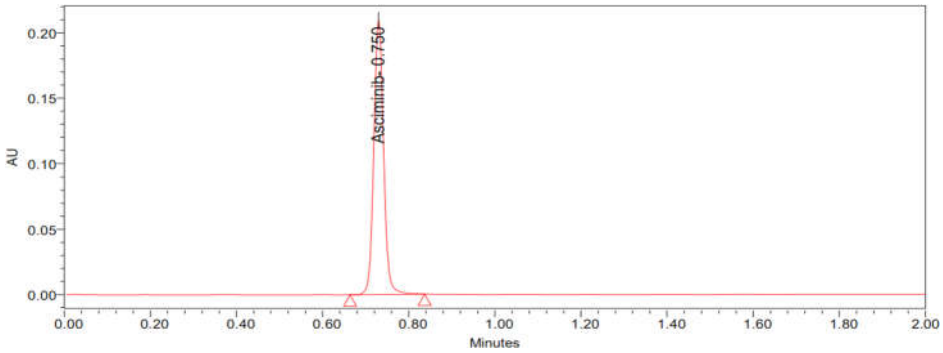

Fig.9: Photolytic degradation chromatogram of asciminib

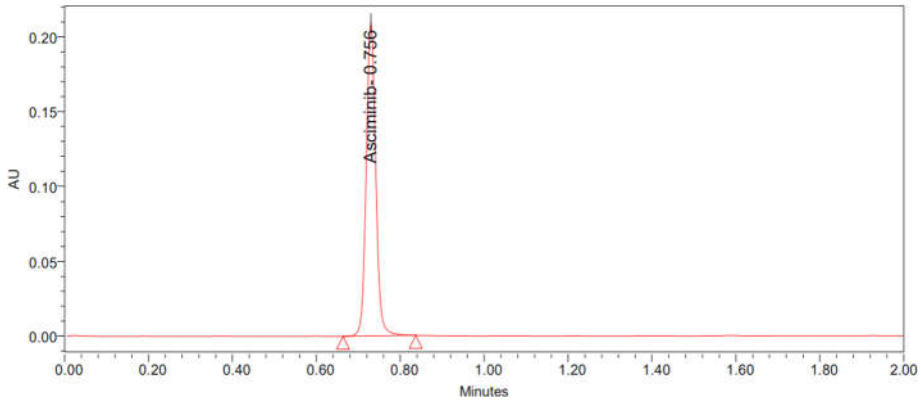

Fig.10: Hydrolyze degradation chromatogram of asciminib

CONCLUSION:

To estimate asciminib in tablet dosage form, a straightforward, accurate, and specific methodology was developed. The retention time for asciminib was recorded at 0.565 minutes. The percent relative standard deviation (RSD) for both method and intermediate precision were found to be 0.29 % and 0.65 %, respectively. The recovery rate for asciminib was determined to be 100.3 %. The limit of detection (LOD) and limit of quantification (LOQ) values for asciminib, derived from regression equations, were 0.11 µg/mL and 0.3 µg/mL, respectively. The regression equation for asciminib is expressed as y = 63691x + 81976, The results from the forced degradation test indicated the presence of degradation peaks under acidic, basic, thermal, and peroxide-stressed conditions. This drug has recently received FDA approval, and no analytical method had been previously reported. The analytical method presented here has successfully minimized both retention time and run time. Consequently, the developed method is rapid, concise, straightforward, and cost-effective, making it suitable for regular quality control assessments in the industry.The future scope of this study includes extending the validated UPLC method to other dosage forms, applying principles of green analytical chemistry, adapting the method for combination therapies, developing bioanalytical methods, and expanding its application to various pharmaceutical formulations.

CONFLICT OF INTEREST:

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

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