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Research Article

Method Development And Validation Of Asciminib By RP-HPLC

Ogale Shital Ashokrao* , Madhav A. Shetkar, S. S. Patil

Maharashtra college of pharmacy, Nilanga, Latur

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ABSTRACT

Another methodology was set up for synchronous estimation of a Asciminib by RP-HPLC system. The chromatographic conditions were viably created for the unit of Asciminib by using Inertsil - ODS C18(250 x 4.6 mm, 5µ), stream is 1.0 ml/min, convenient stage extent was Methanol: Acetonitrile (60:40), recognizable proof wave length was 274nm.

INTRODUCTION

High Performance Liquid Chromatography (HPLC) was derived from the classical column chromatography and is one of the most important tools of analytical chemistry today. In the modern pharmaceutical industry, high-performance liquid chromatography (HPLC) is the major and integral analytical tool applied in all stages of drug discovery, development, and production. HPLC is the method of choice for checking peak purity of new chemical entities, monitoring reaction changes is in synthetic procedures or scale up, evaluating new formulations and carrying out quality control / assurance of the final drug products. The Goal of HPLC method is to try & separate, quantify the main drug, any reaction impurities, all available synthetic intermediates and any degradants. High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantify the compounds that are present in any sample that can be dissolved in a liquid. HPLC is the most accurate analytical methods widely used for the quantitative as well as qualitative analysis of drug product and used for determining drug product stability. HPLC principle is the solution of sample is injected into a column of porous material (stationary phase) and liquid phase (mobile phase) is pumped at higher pressure through the column. The principle of separation followed is the adsorption of solute on

***Corresponding Author:** Ogale Shital Ashokrao
Address: *Maharashtra college of pharmacy, Nilanga, Latur*
Email : ogaleshital99@gmail.com

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stationary phase based on its affinity towards stationary phase.

The technique of HPLC has following features.

- High resolution
- Small diameter, Stainless steel, Glass column
- Rapid analysis
- Relatively higher mobile phase pressure
- Controlled flow rate of mobile phase

HPLC Method Development

Methods are developed for new products when no official methods are available. Alternate methods for existing (Non-Pharmacopoeial) products are to reduce the cost and time for better precision and ruggedness. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available. The goal of the HPLC-method is to try & separate, quantify the main active drug, any reaction impurities, all available synthetic inter-mediates and any degradants.

Steps involved in Method development are

- Understanding the Physicochemical properties of drug molecule
- Selection of chromatographic conditions
- Developing the approach of analysis
- Sample preparation
- Method optimization
- Method validation

MATERIALS AND METHODS

Instruments-Instruments:

- HPLC –Waters Model NO.2690/5 series Compact System Consisting Inertsil-C18 ODS column.
- UV spectrophotometer (Systronics)
- Electronic balance (SARTORIOUS)
- Sonicator(FAST CLEAN)

Substances containing chemicals:

- Methanol HPLC Grade.
- Buffer(KH2PO4)Hplc Grade.

EXPERIMENTAL:

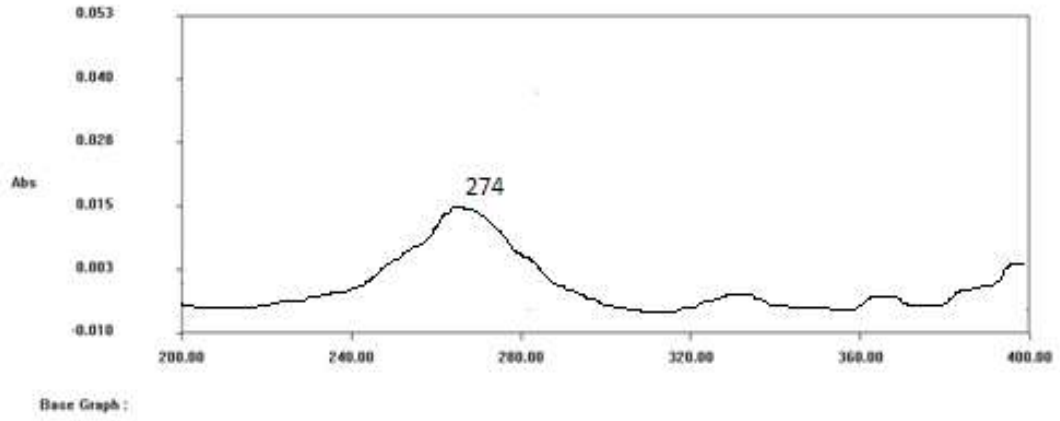
i. Stock and standard Working solution:

ii. Nebivolol is used as working standard in method development

iii. Stock Solution Preparation :Take 100mg Asciminib working standard in 100ml V.F add methanol sonicate it 30minets,(That is 1000ppm solution).

iv. iii. Further Dilution (or) Trails Solution: Take 10ml of above solution in 100ml V.F add Methanol up to mark sonicate it 10minets(That 100ppm solution

v. iv. Selection of wave length: Scan standard solution in UV spectrophotometer between 200 nm to 400 nm on spectrum mode, using diluents as a blank. Asciminib shows λmax at 274nm.



5. Development of an HPLC method :

The goal of this study was to improve the assay technique for simultaneous quantification of Asciminib on literature surveys. As a result, the trials detailed below show how the optimization was accomplished.

Sr. No.	Trial	Mobile Phase	Name of the peak	Retention time (min)	Flow rate	Time to run	Tempo in the column
1.	1	Degassed Methanol: Water 65:35	Asciminib	3.261	1.0ml/min	6min	Ambient
2.	2	Degassed Acetonitrile: methanol 10:90	Asciminib	2.925	1.0ml/min	6min	Ambient
3.	3	Degassed Acetonitrile: methanol 35:65	Asciminib	2.910	1.0ml/min	6min	Ambient

6. Method Validation:

System Suitability:

A Standard solution was prepared by using Asciminib working standard as per test method and was injected Five times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Asciminib, retention times and peak areas.

Table-1: Data Of System Suitability

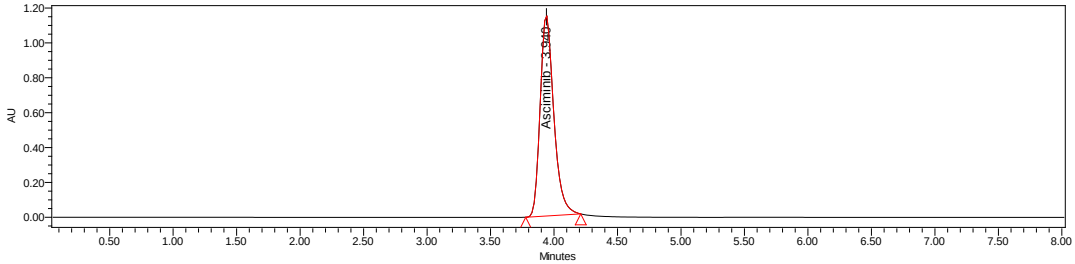
Injection	RT	Peak Area	USP Plate count	USP Tailing
1	3.940	674753	10953.609752	1.153539
2	3.944	674261	10951.014286	1.155271
3	3.941	675298	10003.278630	1.157740
4	3.941	679221	10986.906427	1.159499
5	3.940	688636	10946.878423	1.152820
Mean	3.9412	678433.8	10768.34	1.155774
SD	0.001643	6031.135	-------	-------

% RSD	0.041692	0.888979	-------	-------

Specificity:

Solutions of standard and sample were prepared as per the test method are injected into chromatographic system.

Inference: Got a peak for std at an Rt of 3.940min



Precision

Repeatability:

System precision:

Standard solution prepared as per test method and injected five times.

Method precision:

Prepared five sample preparations individually using single as per test method and injected each solution. % relative standard deviation of individual Nebivolol, from the five units should be not more than 2.0%. The assay of Nebivolol should be not less than 98% and not more than 102.0%.

Intermediate precision (analyst to analyst variability):

A study was conducted by two analysts as per test method. The individual assays of Nebivolol should be not less than 98% and not more than 102% and %RSD of assay should be NMT2.0% by both analysts.

Table-2: Data Of Repeatability

Conc 40ppm	System precision			Method precision			Intermediate precision		
	Inj	**Peak Areas of Asciminib**	**% Assay**	**Inj**	**Peak Areas of Asciminib**	**% Assay**	**Inj**	**Peak Areas of Asciminib**	**% Assay**
	1	671753	101.5	1	663495	100.2	1	666792	100.71
	2	671261	101.4	2	665992	100.6	2	664360	100.34
	3	671298	101.4	3	669828	101.2	3	655696	99.03
	4	670221	101.2	4	661098	99.85	4	664147	100.31
	5	670636	101.3	5	663241	100.2	5	664127	100.30
Statistical Analysis	**Mean**	671033.8	101.36	**Mean**	664730	100.41	**Mean**	663024	100.13
	SD	603.647	0.114018	**SD**	3336.016	0.512567	**SD**	4247.353	0.6425
	% RSD	0.089958	0.112488	**% RSD**	0.502	0.513	**% RSD**	0.641	0.642

Accuracy:

A study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of Asciminib into each volumetric flask for each spike level to get the concentration of Asciminib equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of Asciminib was calculated.

Table-5: Data Of Accuracy

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Injection 1	20	20.04	99.49	**MEAN**	99.44
50% Injection 2	20	19.97	99.46	**%RSD**	0.062802
50% Injection 3	20	20.02	99.37		
100 % Injection 1	40	40.01	101.4	**MEAN**	100.57
100 % Injection 2	40	40.05	100.3	**%RSD**	0.721534
100% Injection 3	40	39.98	100.03		
150% Injection 1	60	60.08	99.81	**MEAN**	99.83
150% Injection 2	60	59.97	99.84	**%RSD**	0.02520
150% Injection 3	60	59.98	99.86		

Linearity:

A Series of solutions are prepared using Asciminib working standard at concentration levels from 20ppm to 70 ppm of target concentration .

LOD AND LOQ (LIMIT OF DETECTION AND LIMIT OF QUANTITATION):

From the linearity plot the LOD and LOQ are calculated:

$$LOD = 3.3\,\sigma/S$$

$$= \frac{3.3 \times 6031.135}{16594} = 1.2$$

$$LOQ = 10\,\sigma/S$$

$$= \frac{10 \times 6031.135}{16594} = 3.6$$

Table6: Data Of Linearity

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	16594
20	328546	y-Intercept	-1648
30	488296	Correlation Coefficient	0.999
40	670413		
50	830308		
60	992582		
70	1158499		

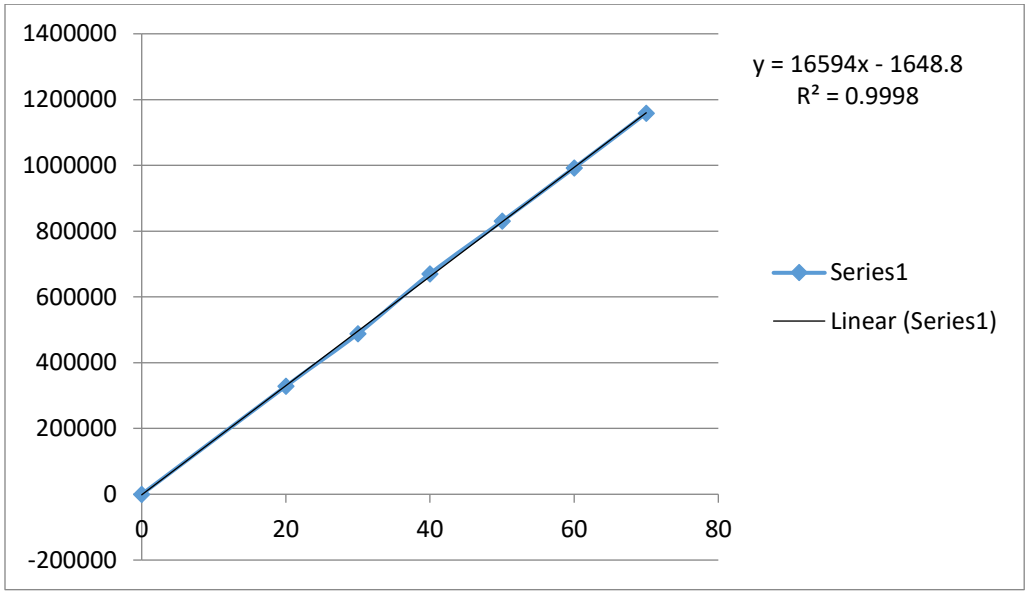


Fig 1: linearity plot(concentration vs respose)

Ruggedness:

System to system variability:

System to system variability study was conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared and each was analyzed as per test method. Comparison of both the results obtained on two different HPLC systems, shows that the assay test method are rugged for System-to-system variability.

Table 7 : Data On System Variability

Sr. No:	Peak area	Assay % of Asciminib
1	664360	100.34
2	664098	100.30
3	665696	100.54
4	663289	100.18
5	664147	100.31
6	663495	100.21
Mean	664180.8	100.3133
%RSD	0.127783	0.126673

Robustness:

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 1.0ml/min and 1.2ml/min. The system suitability parameters were evaluated and found to be within the limits for 1.0ml/min and 1.2ml/min flow. Asciminib was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates 1.0ml/min.

Table: 10 Data For Effect Of Variation In Flow Rate:

Flow 0.8 ml	Std Area	Tailing factor	Flow 1.0 ml	Std Area	Tailing factor	Flow 1.2 ml	Std Area	Tailing factor
	620286	1.322089		664322	1.604878		602077	1.285372
	619282	1.331920		665792	1.584354		601854	1.319385
	621337	1.296438		664360	1.543805		602403	1.292055
	620456	1.315454		665696	1.568590		603421	1.304561

Avg	620765	1.326551	**Avg**	663147	1.559986	**Avg**	602465	1.294621
SD	620425.2	1.31849	**SD**	664663.4	1.572323	**SD**	602444	1.299199
%RSD	754.0018	0.013728	**%RSD**	1100.917	0.023367	**%RSD**	599.8833	0.013223

Market Sample:

Drug name	Brand name	Company
Asciminib	Scemblix	Novartis

$$\% \text{ Assay} = \frac{\text{Amount found(x)}}{\text{Amount added}} \times 100$$

X=y-c/m

Injection	Peak Areas of Asciminib	%Assay
1	658795	99.50
2	659678	99.63
3	655692	99.03
4	659382	99.59
5	660584	99.77
Mean	658826.2	99.504
SD	1867.386	0.282276
% RSD	0.283441	0.283684

FTIR

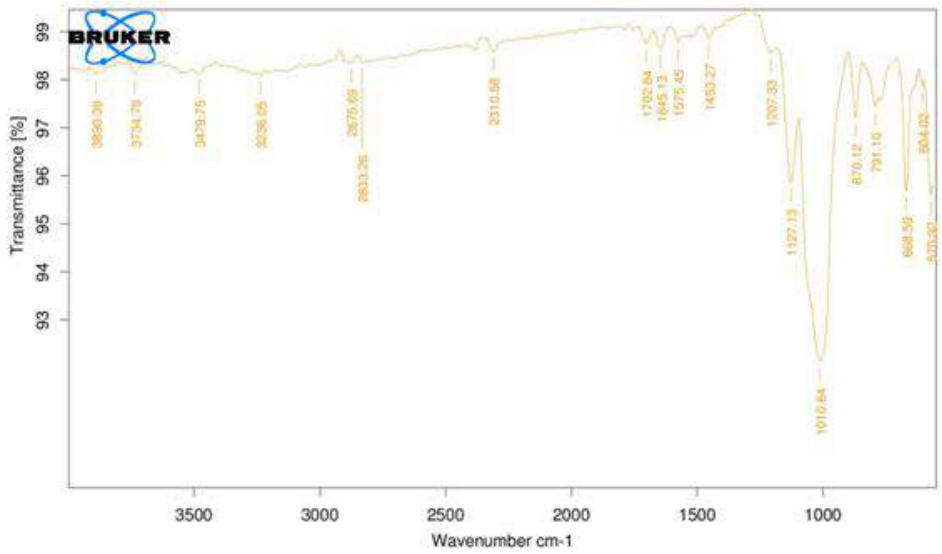


Fig 2: FTIR Spectra For Asciminib

SUMMARY AND CONCLUSION:

Different parameters were studied to create the analytical approach. For starters, the maximum

absorbance of Asciminib was discovered to be 274nm. The injection volume was set at 20µl, which resulted in a nice peak area. The Inertsil C18 column was employed in this work, and ODS picked a nice peak shape. The temperature of the ambient environment was determined to be adequate for the type of the medication solution. Because of the good peak area, adequate retention duration, and good resolution, the flow rate was set at 1.0ml/min. Different mobile phase ratios were investigated, however the mobile phase with a Methanol: Acetonitrile (60:40) ratio was chosen because to its symmetrical peaks and high resolution. As a result, the planned research made use of this mobile phase. The accuracy of both the system and the procedure was determined to be precise and well within range. The correlation coefficient and curve fitting were discovered during the linearity investigation. For both medicines, the analytical approach was shown to be linear throughout a range of 20-70ppm of the target concentration. Both robustness and ruggedness tests were passed by the analytical. The relative standard deviation in both circumstances was excellent.

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