



Research Paper

Development and validation of Abrocitinib In RP- HPLC

Kotla.S.M.Chaitanya*, V.Jagadhathri¹, P. Harshitha¹,G.Sindhuja¹, B. Sumathi¹

* Associate professor, Department of Pharmaceutical analysis, A.K.R.G.College of Pharmacy, Affiliated to JNTUK, Nallajerla, East godavari, Andhra Pradesh, 534112

¹ Department of Pharmaceutical analysis, A.K.R.G.College of Pharmacy, Affiliated to JNTUK, Nallajerla, East godavari, Andhra Pradesh, 534112

ARTICLE INFO

ARTICLE HISTORY

Received 21 March 2024

Received in revised form 22 March 2024

Accepted 25 March 2024

Available online- 31 March 2024

Key words:

Abrocitinib, RP-HPLC

ABSTRACT

A new selective reliable analytical RP-HPLC method for estimating Abrocitinib in bulk and marketed dosage form was developed and validated. The chromatographic analysis was carried on a reverse phase analytical column of Inertsil C-18 (250mm×4.6mm,5μm) column with a mobile phase of phosphate buffer PH 4.8:Methanol(55:45% v/v) at a flow rate of 1ml per min. At a wavelength of 282nm. The desired analyte was identified without any interference. The retention time of the drug was discovered to be 4.8min. The graphical method produced linear response with regression co-efficient of 0.99 for the concentration range of 10-130μgm/ml. The LOD and LOQ values were 1.3 and 3.9μgm/ml, respectively. These results obtained, showed a good agreement with the declared content that of the formulation. Therefore, the proposed method is rapid, accurate and validated for the quantification of Abrocitinib in solid dosage forms.

Corresponding author :

Mr. Kotla. S. M chaitanya ,Department of Pharmaceutical analysis, A.K.R.G.College of Pharmacy, Affiliated to JNTUK, Nallajerla, East godavari, Andhra Pradesh, 534112
EMAIL: akrgpharmacycollege@gmail.com



1. INTRODUCTION

HPLC is in like manner alluded to as unreasonable pressure fluid chromatography considering unnecessary strain is utilized to improvement the skim price and inexperienced division through compelling the telephone phase through at tons higher price. The stress is finished the usage of a siphoning device¹. The improvement of HPLC from old fashion phase chromatography is probably ascribed to the improvement of greater modest molecule sizes. greater modest molecule length is critical considering they provide extra noteworthy floor district over the traditional sizeable molecule sizes. The primary objective of proposed work is to develop new simple, sensitive, accurate and economical analytical method for the estimation of Abrocitinib in bulk and marketed pharmaceutical dosage form².

Abrocitinib drug belongs to class called JANUS KINASE (JAK) inhibitors. It works by Decreasing the activity of the immune system.

Abrocitinib 100mg once daily can be considered for long term maintainance. It is P-Glycoprotein Inhibitor. It is orally available.

The mechanism of action of Abrocitinib is as a Janus Kinase Inhibitor, and P-Glycoprotein Inhibitor. Inhibits cytokine induced phosphorylation of STAT By JAK pairs that include JAK1 signalling by JAK2/JAK2.

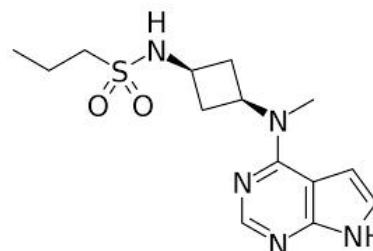


Figure no. 1 Structure of Abrocitinib

2.MATERIALS AND METHOD

Chemicals

S.No	Chemical	Brand names
1	Abrocitinib (Pure)	Cibinqo
2	Water for HPLC	Lichrosolv (merck)
3	Acetonitrile for HPLC	Merck

Instrumentation

The HPLC system consisted of WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA Detector. consisting of gradient pump having a flow range from 0.2 up to 10 ml/min and Inertsil octa decyl silane (150X4.6X5 μ) C- 18 column was used⁴. The HPLC system was equipped with the Software having double-beam photometer with light source deuterium lamp and wavelength limit of 190-600 nm having integrated degasser⁵. The Mobile phase was a composite of buffer (pH-5) and Methyl alcohol in the ratio of 20:80 for Abrocitinib. Flow rate was maintained at 1ml/minute. Wavelength for the chromatographic separation used was 306 nm. Injection volume was maintained at 20 μ l. The column was maintained at ambient temperature. The mobile phase was purified by passing through 0.45 μ m micron filter prior to use.

Preparation of Reagents and Standards

Mobile phase selection

Standard stock solution of Abrocitinib was prepared by dissolving 20 mg of Abrocitinib standard into 100 ml of methanol which gives 200 μ g/ml of standard stock solution. From this solution 1ml was taken and diluted it with 10 ml of mobile phase (mobile phase which used for trials) to give 20 μ g /ml standard working solution⁶. Above working standard preparation was injected for mobile phase selection. Different mobile phase combinations was used for the study until system suitability parameters was obtained.

Materials

Abrocitinib reference standard was furnished by the sunpharm Research Centre, mumbai. The pharmaceutical dosage form of Abrocitinib, Aluminium hydroxide, Abrocitinib, Alu-Cap, was procured from local pharmacy with a 320 mg label claim, manufactured by pharma force lab paonta sahib, India. All chemicals used was of HPLC grade, Methanol and water both HPLC grade, was from Merck, potassium hydroxide and potassium dihydrogen phosphate of analytical grade from Merck.

Procedure For Buffer Preparation (Phosphate Buffer, pH 5)-

Dissolve 6.8 g of potassium dihydrogen phosphate in 1000 ml of water and adjust the pH to 5.0 with 10M potassium hydroxide.

Method validation

Linearity and Range- Standard stock solution of Abrocitinib was prepared by dissolving 20 mg of Abrocitinib standard into 100 ml of methanol which gives 200 μ g/ml of standard stock solution. Method linearity was displayed over the concentration range of 10 μ g /ml to 30 μ g /ml. Concentration range of 10 μ g /ml to 30 μ g /ml was obtained by dissolving 0.5ml, 0.75ml, 1ml, 1.25 ml, 1.5 ml of Abrocitinib standard stock solution with mobile phase diluted with 10 ml to obtain 10 μ g /ml, 15 μ g/ml, 20 μ g/ml, 25 μ g/ml, 30 μ g/ml of final concentration of Abrocitinib for linearity determination.

Precision Studies of Abrocitinib -Precision studies was carried out in three different ways as intraday precision, interday precision and repeatability. Intraday precision studies was conducted for three different concentrations at three times each for single day and interday precision studies was conducted for three different concentrations at three times at alternate days respectively and repeatability for one concentration at six times⁷.

Recovery/accuracy studies- Recovery studies was performed by spiking the sample with the standard and then finding out the recovery at 80,100,120 percent. In this an chunk of standard drug was mixed to the sample preparation and find out recovered amount from sample preparation as % recovery.

Procedure: Standard Stock Solution of Abrocitinib - Standard stock solution of Abrocitinib was made by dissolving 20 mg of standard drug in 100 ml of Methyl alcohol to make 200 μ g/ml solution.

Standard Working Solution for Recovery (20 μ g/ml) for Abrocitinib:

1ml of standard stock solution of Abrocitinib was taken and diluted with 10 ml of mobile phase.

Sample Stock Solution of Abrocitinib: Weigh and powdered 20 tablets. Take tablet powder equivalent to 20 mg of Abrocitinib in to a 100ml volumetric flask. Add 60 ml Methanol. Shake for 15 minutes and sonicate for 10 minutes. Make up volume with Methanol. Filter this solution through 0.45 μ m micron filter prior to use to give 200 μ g/ml⁸.

Sample Solution for Recovery for Abrocitinib (20 μ g/ml)

Take 1ml from sample stock solution and then diluted with 10 ml of mobile phase.

Recovery Solution Preparation for Abrocitinib- Recovery solution was prepared for standard by dissolving 0.8ml, 1ml and 1.2ml of standard Abrocitinib and then volume make upto 10 ml with the diluent. Recovery solution of sample was prepared by dissolving 1ml from sample stock solution and then diluted with 10ml of mobile phase to give 20 μ g/ml. After that injected standard solution for recovery, sample solution for recovery and recovery solutions of 80%, 100% and 120% and then calculated % recovery⁹.

3. RESULT AND DISCUSSION

SNO	Peak name	RT	Area ($\mu V \cdot \text{sec}$)	Height (μV)	USP plate count	USP Tailing
1	Abrocitinib	4.865	856985	69854	8569	1.26
2	Abrocitinib	4.838	856857	68954	8547	1.25
3	Abrocitinib	4.846	857894	68975	8596	1.25
4	Abrocitinib	4.844	857468	69854	8541	1.26
5	Abrocitinib	4.838	854785	69856	8616	1.25
Mean			856797.8			
Std.dev			1197.992			
%RSD			0.139822			

Table no.1 Results of system suitability for Abrocitinib

Specificity of drug

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Abrocitinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 2ml of the above Abrocitinib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Preparation of Sample Solution:

Take average weight of the Powder and weight 10 mg equivalent weight of Abrocitinib sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 2ml of the above Abrocitinib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

S.no.	Peak name	RT	Area ($\mu V \cdot \text{sec}$)	Height (μV)	USP plate count	USP tailing
1	Abrocitinib	4.838	866854	70152	8659	1.26
2	Abrocitinib	4.840	868789	69987	8657	1.27
3	Abrocitinib	4.838	865987	70154	8654	1.26
4	Abrocitinib	4.842	865896	69985	8659	1.27
5	Abrocitinib	4.846	859864	69587	8674	1.27
Mean			865415.8			
Std.dev			3272.034			
%RSD			0.378088			

Table no.2 Results of Assay (Standard) for Abrocitinib

Linearity

Accurately weigh and transfer 10 mg of Abrocitinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and

sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Procedure:

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

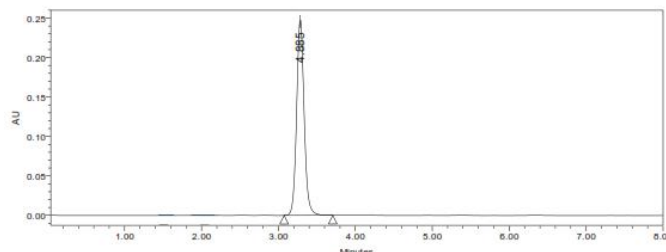


Figure no. 2 Chromatogram showing linearity level

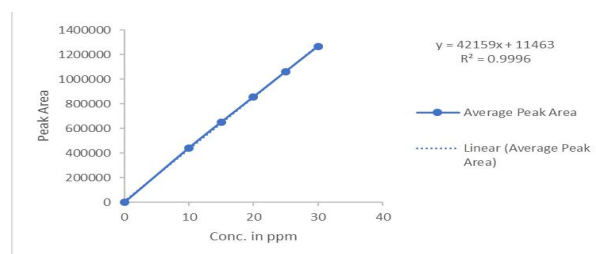


Figure no. 3 Calibration Curve of Abrocitinib

Preparation of Abrocitinib Product Solution for Precision

Accurately weigh and transfer 10 mg of Abrocitinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Take 2ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Sno	peakname	Retention time	Area ($\mu V \cdot \text{sec}$)	Height (μV)	Usp plate count	Usp tailing
1	Abrocitinib	4.840	856985	69856	8569	1.26
2	Abrocitinib	4.842	856898	69845	8597	1.25
3	Abrocitinib	4.834	856789	69865	8598	1.26
4	Abrocitinib	4.838	854789	69874	8569	1.25
5	Abrocitinib	4.846	854789	69898	8564	1.26
6	Abrocitinib	4.844	856978	69859	8599	1.25
Mean			857048.8			
Std.dev			1617.106			
%RSD			0.188683			

Table no.3 Results of precision for Abrocitinib

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

sno	peak name	RT	Area (µg/ml)	Height (µV)	USP Plate count	USP Tailing
1	Abrocitinib	4.882	865845	70023	8659	1.27
2	Abrocitinib	4.877	865835	70015	8667	1.27
3	Abrocitinib	4.877	867584	69989	8654	1.28
4	Abrocitinib	4.882	865987	70114	8567	1.28
5	Abrocitinib	4.877	865975	69985	8635	1.27
6	Abrocitinib	4.877	865982	69998	8695	1.28
			685954.8			
			1022.223			
			0.118046			

Table no.4 Results of linearity for Abrocitinib

Accuracy

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Abrocitinib and calculate the individual recovery and mean recovery values.

Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated.

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	429549.7	10	9.916	99.16%	99.68%
100%	856189.3	20	20.036	100.18%	
150%	1272534	30	29.912	99.706%	

Table no.5 The accuracy results for Abrocitinib

Robustness

The robustness was performed for the flow rate variations from 0.9 mL/min to 1.1 mL/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Abrocitinib. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase $\pm 5\%$. The standard and samples of Abrocitinib were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	856985	4.865	8547	1.25
Less Flow rate of 0.9 mL/min	841542	5.021	8256	1.23
More Flow rate of 1.1 mL/min	812546	3.997	8146	1.20
Less organic phase	802654	6.242	8365	1.16
More organic phase	826549	4.002	8154	1.14

Table no.6 Results for Robustness

4. CONCLUSION

Thus the RP-HPLC method developed for abrocitinib is in adherence to all the parameters such as linearity, accuracy, precision, reproducibility and specificity¹⁰⁻¹¹. As apparent from the validation results. All of the checked parameters are in accordance with the ICH guidelines. Estimation of regression values, RSD (%) and standard deviation gives a multi faceted approach to the developed method. Thus making it very adaptable in routine usage. Hence the developed RP-HPLC technique can be utilised for the standard analysis of abrocotinib drug in bulk and marketed formulation¹²⁻¹³.

5.CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

6. ACKNOWLEDGEMENT

We are highly grateful to our Honorable Chairman, xxxcollege of Pharmacy, Telangana state for providing the necessary facilities to carry out this research work.

7. REFERENCE

1. R. B. Pandhare, R. N. Kulkarni, V. K. Deshmukh, P. B. Mohite, and A. R. Pawar, "High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery," *J. Pharm. Biol. Sci.*, 2021, doi: 10.18231/j.jpbs.2021.002.
2. M. Attimarad, K. K. Mueen Ahmed, B. E. Aldhubaib, and S. Harsha, "High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery," *Pharm. Methods*, 2011, doi: 10.4103/2229-4708.84436.
3. R. Deepthi, "INSTRUMENTATION AND FUTURE PROSPECTS OF HPTLC- A REVIEW," *World J. Pharm. Res.*, 2021.
4. C. Su, Y. Liu, Y. He, and J. Gu, "Analytical methods for investigating in vivo fate of nanoliposomes: A review," *Journal of Pharmaceutical Analysis*. 2018. doi: 10.1016/j.jpha.2018.07.002.
5. J. Shah and R. Kotadiya, "A Critical Review on Analytical Methods for Recently Approved FDC Drugs: Pregabalin and Etoricoxib," *Critical*

-
- Reviews in Analytical Chemistry. 2022. doi: 10.1080/10408347.2020.1855411.
6. B. Ramu and K. B. Chittela, "High Performance Thin Layer Chromatography and its role in the Pharmaceutical Industry: Review," *Sci. J. Biosci. Bioeng.*, 2018.
7. F. Hameedat, S. Hawamdeh, S. Alnabulsi, and A. Zayed, "High Performance Liquid Chromatography (HPLC) with Fluorescence Detection for Quantification of Steroids in Clinical, Pharmaceutical, and Environmental Samples: A Review," *Molecules*. 2022. doi: 10.3390/molecules27061807.
8. P. Y. G. Rao, B. Sreenivasa Rao, K. V. N. Suresh Reddy, and C. Sudhakar, "Quantification of related/degraded impurities in tolcaptan drug substance by HPLC," *Int. J. Res. Pharm. Sci.*, 2020, doi: 10.26452/ijrps.v11i3.2641.
9. A. Abdu Hussen, "High-Performance Liquid Chromatography (HPLC): A review," *Ann. Adv. Chem.*, 2022, doi: 10.29328/journal.aac.1001026.
10. I. BULDUK, "HPLC-UV method for quantification of favipiravir in pharmaceutical formulations," *Acta Chromatogr.*, 2021, doi: 10.1556/1326.2020.00828.
11. Boukhobza and D. C. Crans, "Application of HPLC to measure vanadium in environmental, biological and clinical matrices," *Arabian Journal of Chemistry*. 2020. doi: 10.1016/j.arabjc.2017.10.003.
12. A. M. Sabir, M. Moloy, and P. S. Bhasin, "HPLC METHOD DEVELOPMENT AND VALIDATION: A REVIEW," *Int. Res. J. Pharm.*, 2016, doi: 10.7897/2230-8407.04407.
13. S. K. Bhardwaj, K. Dwivedi, and D. D. Agarwal, "A Review: HPLC Method Development and Validation," *Int. J. Anal. Bioanal. Chem.*, 2015.