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Quality by Design Assisted Chromatographic Method Development and Validation for Estimating Abrocitinib in Bulk and Dosage Forms by RP-HPLC Technique

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Abstract
A new method was established for simultaneous estimation of Tinidazole and Diloxanide furoate by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Tinidazole and Diloxanide furoate by using Thermosil RP C18 (4.5×100 mm) 5.0µm, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol. The retention times were found to be 2.408mins and 3.016mins. The % purity of Tinidazole and Diloxanide furoate was found to be 99.24% and 101.27% respectively. The system suitability parameters for Tinidazole and Diloxanide furoate such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.2. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study Tinidazole and Diloxanide furoate was found in concentration range of 50ppm-250ppm and 5ppm-25ppm and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 100.56% and 101.47%, %RSD for repeatability was 0.1 and 0.3, % RSD for intermediate precision was 0.19 and 0.57respectively. The precision study was precise, robust, and repeatable. LOD value was 4.27 and 6.80, and LOQ value was 0.0272 and 0.3125 respectively.

Keywords: Thermosil RP C18 column, Tinidazole and Diloxanide furoate, RP-HPLC

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Contents:

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1. Introduction

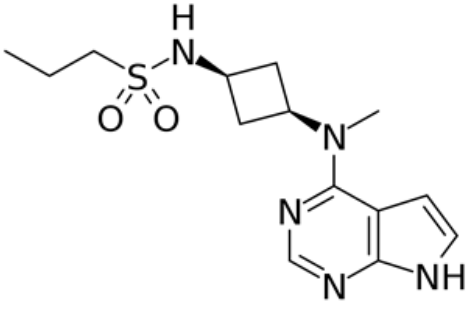

Figure 1: Abrocitinib

Abrocitinib undergoes CYP-mediated oxidative metabolism. CYP2C19 is the predominant enzyme, accounting for about 53% of drug metabolism. CYP2C9 is responsible for 30% of drug metabolism. About 11% and 6% of the drug is metabolized by CYP3A4 and CYP2B6, respectively. In a human radiolabeled study, the parent drug was the most prevalent circulating species.

IUPAC Name : N-[3-[methyl (7H-pyrrolo [2,3-d]pyrimidin-4-yl)amino] cyclobutyl]propane-1-sulfonamide
Mol Formula : C14H21N5O2S

Molecular Weight : 323.42 g/mol
Category : Janus kinase inhibitor
Solubility : Soluble in water.
Melting Point : ~189 °C
pKa : 6.45
Protein Binding : Approximately 64%, 37% and 29% of circulating abrocitinib and its active metabolites M1 and M2, respectively, are bound to plasma proteins.

2. Materials and Methods

Wave length selection:

UV spectrum of 10 µg / ml Abrocitinib in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 290. At this wavelength both the drugs show good absorbance.

Optimization of Column:

Inersil ODS(4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1.5 ml/min flow.

Optimized chromatographic conditions:

Instrument used : High performance liquid chromatography equipped with Auto Sampler and DAD or UV detector
Temperature : Ambient
Column : Inertsil ODS C_{18} (4.6 x 150mm, 5.0µm)
Buffer : Ortho phosphoric acid buffer
Mobile phase : 30% Buffer: 60% Acetonitrile: 10% Methanol
Flow rate : 1.5 ml per min
Wavelength : 290 nm
Injection volume : 20 µl
Run time : 4min.

PREPARATION OF BUFFER AND MOBILE PHASE:

Preparation of 0.1% Ortho phosphoric acid buffer:

Pipetted 1 ml of ortho phosphoric acid in 1000 ml HPLC water.

Preparation of mobile phase:

Mix a mixture of above buffer 300ml (30%), 600 ml Acetonitrile (60%) and 100ml Methonol HPLC (10%) and degas in ultrasonic water bath for 5 minutes. Filter through 045 µ filter under vacuum filtration.

Diluent Preparation: Use the Mobile phase as Diluents.

Validation parameters:

1. ASSAY:

Standard Solution Preparation:

Accurately weigh and transfer 10mg of Abrocitinib working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 10mg of Abrocitinib equivalent weight of the sample into a 10ml clean dry volumetric flask 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 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure:

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for the Abrocitinib peaks and calculate the % Assay by using the formulae.

2. Linearity:

Preparation of stock solution:

Accurately weigh and transfer 10mg of Abrocitinib working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Preparation of Level – I (10ppm of Abrocitinib):

1ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – II (20ppm of Abrocitinib):

2ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – III (30ppm of Abrocitinib):

3ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – IV (40ppm of Abrocitinib):

4ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – V (50ppm of Abrocitinib):

5ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

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.

3. Precision:

Preparation of stock Solution:

Accurately weigh and transfer 10mg of Abrocitinib working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure:

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.

4. Intermediate Precision/Ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day within the laboratory.

Preparation of stock solution: Accurately weigh and transfer 10mg of Abrocitinib working standard into a 10ml

clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure:

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.

5. Accuracy:

For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same.

Preparation of Standard stock solution:

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

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):

Accurately weigh and transfer 5mg of Abrocitinib working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

For preparation of 100% solution (With respect to target Assay concentration):

Accurately weigh and transfer 10mg of Abrocitinib working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

For preparation of 150% solution (With respect to target Assay concentration):

Accurately weigh and transfer 15mg of Abrocitinib working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure:

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Abrocitinib and calculate the individual recovery and mean recovery values.

6. Limit of Detection:

Preparation of Abrocitinib solution:

Preparation of 0.06µg/ml solution:

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

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 0.5ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent. Further pipette 0.4ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

7. Limit of Quantification:

Preparation of Abrocitinib solution:

Preparation of 0.18µg/ml solution:

Accurately weigh and transfer 10mg of Abrocitinib working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent. Further pipette 0.6ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

8. Robustness:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

- The flow rate was varied at 1.4 ml/min to 1.6ml/min.
- Standard solution 30 µg/ml of Abrocitinib prepared and analysed using the varied flow rates along with method flow rate.
- The Organic composition in the Mobile phase was varied from 63% to 77%

Standard solution 30 µg/ml of Abrocitinib was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

Forced Degradation Studies

Accurately weigh and transfer 10mg of Abrocitinib working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Acid degradation:

Pipette 3 ml of Abrocitinib of the above stock solution into a 10ml volumetric flask added about 3 ml of 0.1N Hcl and sonicated for 10minutes and kept it in darkness for 12 hours then refluxed under heat at 60 degrees in a heating mantle for 1 hours. Neutralized the sample solution using 0.1N NaOH and diluted up to the mark with diluents. The Final Sample was filtered through 0.44 micron Injection filters and injected into HPLC system.

Base Degradation:

Pipette 3 ml of Abrocitinib of the above stock solution into a 10ml volumetric flask added about 3 ml of 0.1N NAOH and sonicated for 10minutes and kept it in darkness for 12 hours then refluxed under heat at 60 degrees in a heating mantle for 1 hours. Neutralized the sample solution using 0.1N HCL and diluted up to the mark with diluents. The Final Sample was filtered through 0.44 micron Injection filters and injected into HPLC system.

Thermal Degradation:

Pipette 3 ml of Abrocitinib of the above stock solution into a 10ml volumetric flask and kept in oven under heat at 105 degrees for 12 hours and diluted up to the mark with diluents. The Final Sample was filtered through 0.44 micron Injection filters and injected into HPLC system.

Peroxide Degradation:-

Pipette 3 ml of Abrocitinib of the above stock solution into a 10ml volumetric flask added about 3ml of 3% Hydrogen Peroxide (H_2O_2) and sonicated for 10 minutes and kept in darkness for 12 hours and refluxed under heat at 60 degrees in a heating mantle for 1 hours. The Final Sample was filtered through 0.44 micron Injection filters and injected into HPLC system.

Photo Degradation:-

Pipette 3 ml of Abrocitinib of the above stock solution into a 10ml volumetric flask added about and kept in darkness for 12 hours. The Final Sample was filtered through 0.44 micron Injection filters and injected into HPLC system.

3. Results and Discussions

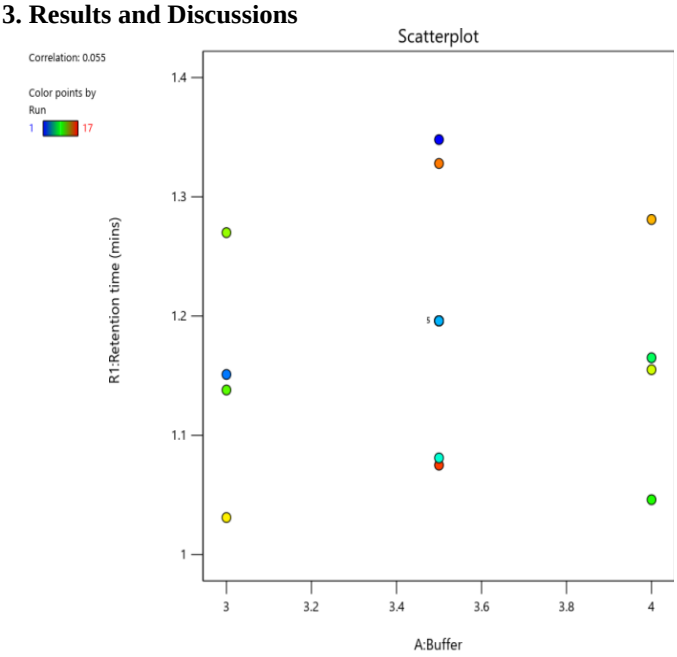

Figure 2

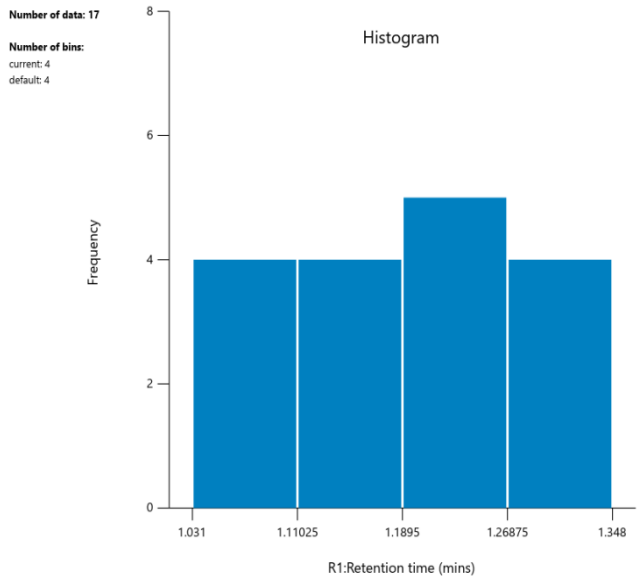

Figure 3

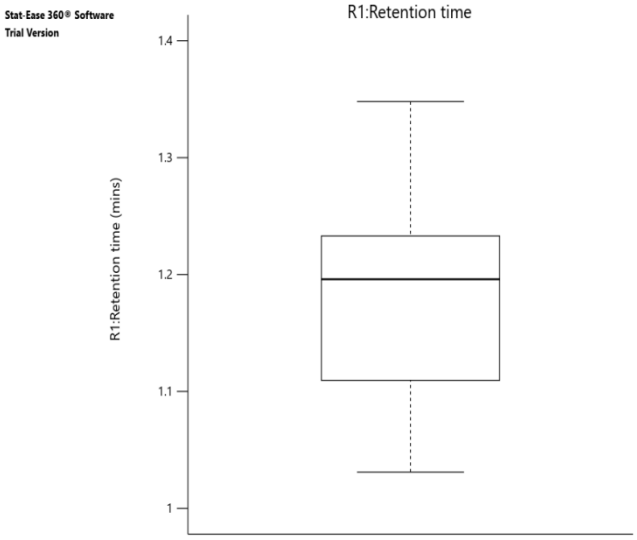

Figure 4

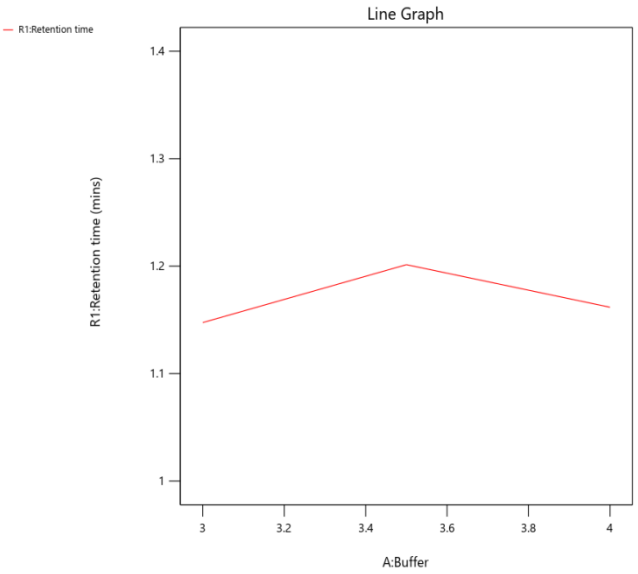

Figure 5

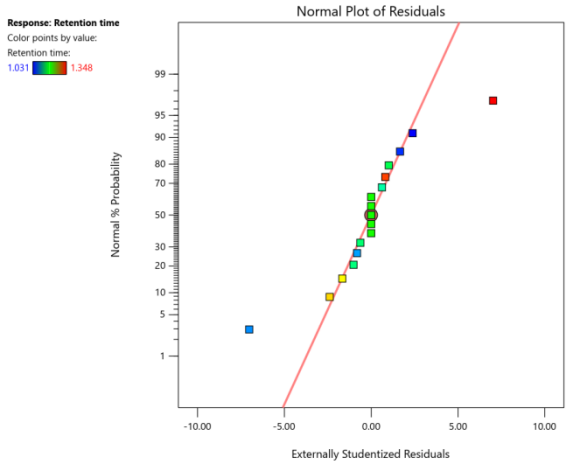

Figure 6

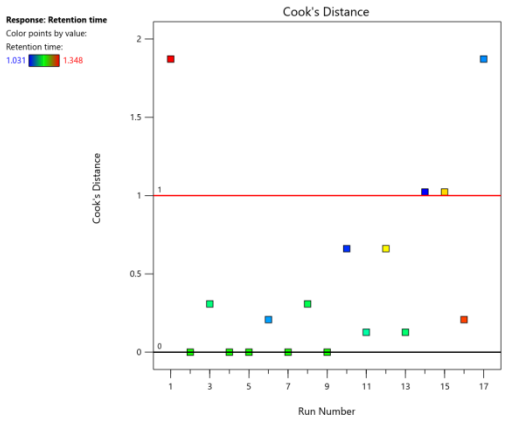

Figure 7

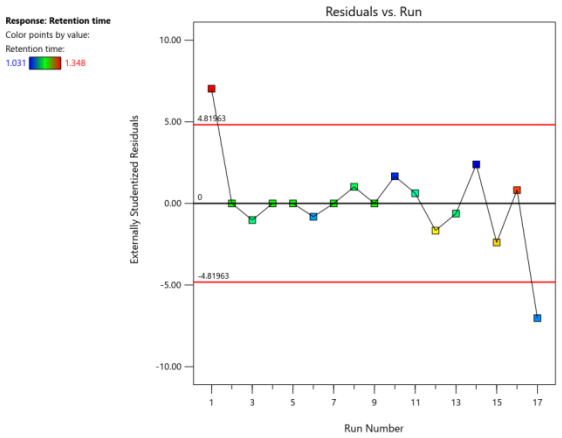

Figure 8

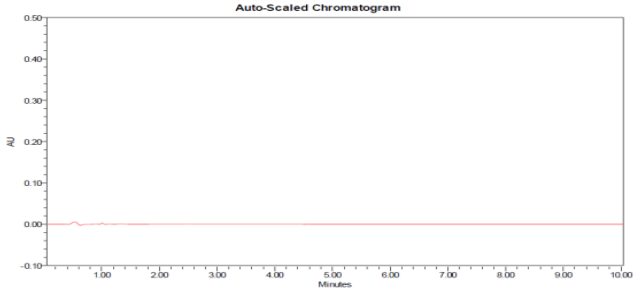

Figure 9: Chromatogram for system suitability

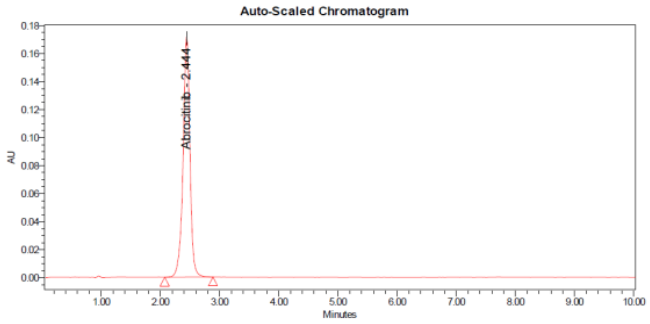

Figure 10: Chromatogram for Standard

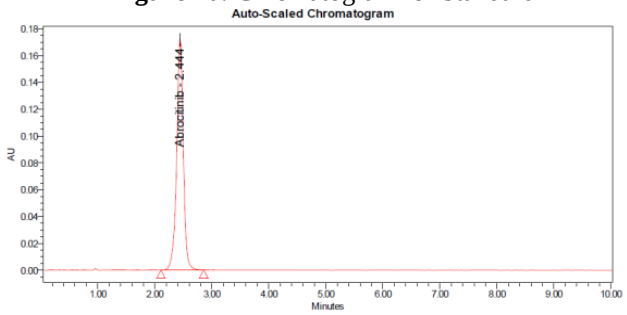

Figure 11: Chromatogram for Sample

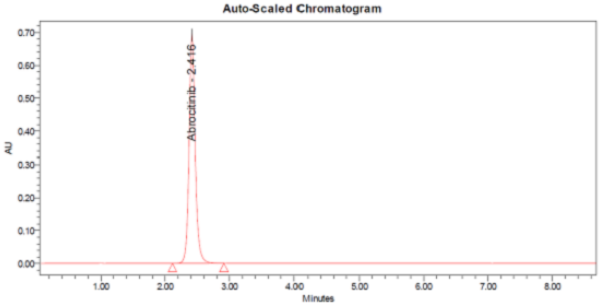

Figure 12: Chromatogram for Linearity

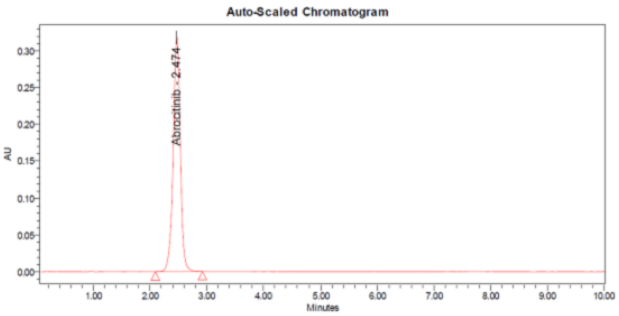

Figure 13: Chromatogram for Precision

Table 1: Results of Precision for Abrocitinib

Injection	Area
Injection-1	2820068
Injection-2	2806192
Injection-3	2803181
Injection-4	2805321
Injection-5	2810736
Injection-6	2806028
Average	2808587.8
Standard Deviation	6141.9
%RSD	0.2

Table 2: Results of system suitability parameters

S.No	Name	RT(min)	Area (µV sec)	Height (µV)	USP tailing	USP plate count
1	Abrocitinib	2.144	1336390	100298	1.75	2502.76

Table 3: Results of Intermediate precision for Abrocitinib

Injection	Area
Injection-1	2820068
Injection-2	2806192
Injection-3	2803181
Injection-4	2805321
Injection-5	2810736
Injection-6	2806028
Average	2808587.8
Standard Deviation	6141.9
%RSD	0.2

Table 4: Accuracy (recovery) data for Abrocitinib

%Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1387761.8	5	5.07	101.43	100.50
100%	2842114.8	10	9.92	99.21	
150%	4251422.6	15	15.13	100.88	

Table 5: Analytical performance parameters of Abrocitinib

Parameters	Abrocitinib
Slope (m)	41811
Intercept (c)	72856
Correlation coefficient (R^2)	0.999

Table 6: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Abrocitinib	61	181	2.97

Table 7: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Abrocitinib	61	608	9.97

Table 8: Results for variation in flow for Abrocitinib

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	1.4	2491.77	1.55
2	1.5	2502.76	1.75
3	1.6	2374.78	1.32

Table 9: Results for variation in mobile phase composition for Abrocitinib

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	1456.04	1.23
2	*Actual	2502.76	1.75
3	10% more	1395.08	1.18

4. Conclusion

The estimation of Abrocitinib was done by RP-HPLC. The assay of Abrocitinib was performed with tablets and the % assay was found to be 99.76 which shows that the method is useful for routine analysis. The linearity of Abrocitinib was found to be linear with a correlation coefficient of 0.999 which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.6 for Abrocitinib which shows that the method is precise. The acceptance criteria of intermediate precision

is RSD should be not more than 2.0% and the method show precision 0.4 for Abrocitinib which shows that the method is repeatable when performed in different days also. The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 100.50% for Abrocitinib. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The acceptance criteria for LOD and LOQ is 3 and 10. The LOD and LOQ for Abrocitinib was found to be 2.97 and 9.97. The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

Conflict of Interest

We affirm that there are no conflicts of interest.

5. References

[1] Becket and Stenlake, Practical pharmaceutical chemistry, part 24th edition CBS publications and distributors, 2005.

[2] P.D. Sethi, HPLC quantitative analysis of pharmaceutical formulations CBS publications and distributors, 1st edition, 2001.

[3] B.K Sharma, instrumental method of chemical analysis, 23rd edition, goal publishers 2004.

[4] International conference on harmonization: ICH Q 2 (R1) Validation of Analytical Procedures: Text and Methodology 1995.

[5] Skoog D A, West D M, Holler FJ: Introduction of analytical chemistry. Sounder college of publishing, Harcourt Brace college publishers. (1994), PP 1-5.

[6] Sakambari Tripathy, Daria Wentzel Validation of enantioseparation and quantitation of an active metabolite of abrocitinib in human plasma First draft submitted: 11 June 2021; Accepted for publication: 17 September 2021; Published online: 4 October 2021 ISSN 1757-6180.

[7] Ellen Q.Wang , phD, Vu Le, Effects of Hepatic Impairment on the Pharmacokinetics of Abrocitinib and Its Metabolites The Journal of Clinical Pharmacology / Vol 61 No 10 2021.

[8] Roya Mohammadi-Meyabadi, Negar Beirampour, Nurria Garros Assessing the solubility of Baricitinib and Drug uptake in different tissues using absorption and Fluorescence spectroscopies journal published on 4 December 2022;

[9] Md Anik Alam, Yang Angela Liu An angle and robust in-line NIR potency deviation detection method for monitoring and control of a continuous direct compression process. International journal of pharmaceutics volume 601, 15 May 2021, 120521.

[10] Reza kamyar, David Lauri pla, Anas Husain, Giuseppe cogoni soft sensor for real-time estimation of tablet potency in continuous direct compression manufacturing operation, international journal of phartmaceutics volume 602, 1 june, 120624.

[11] Elisabetta scali, Domenica scumasi, Jessica ceramella impact of cytochrome P450 enzymes on the phase I metabolism of drugs. Published 15 May 2023.

[12] Tripathy S, Wentzel D, Wan XK, Kavetska O: Validation of enantioseparation and quantitation of an active metabolite of abrocitinib in human plasma. Bioanalysis. 2021, 13(19): 1477-1486.

[13] He H, Guttman-Yassky E: JAK Inhibitors for Atopic Dermatitis: An Update. Am J Clin Dermatol. 2019, 20(2):181-192.