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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF ABROCITINIB IN BULK AND TABLET DOSAGE FORM

[1]*Ms. Manasi S. Wagh, [2]Mr. Rajesh G. Jadhao, [3]Dr. Parag R. Patil

[1]Department of Pharmaceutical Quality Assurance, Kai. Yashodabai Dagadu Saraf Charitable Trust's College of Pharmacy, Sakegaon, Jalgaon, India.
[2]Assistant Professor, Department of Pharmaceutical Chemistry, Kai. Yashodabai Dagadu Saraf Charitable Trust's College of Pharmacy, Sakegaon, Jalgaon, India.
[3]Professor, Principal Kai. Yashodabai Dagadu Saraf Charitable Trust's College of Pharmacy, Sakegaon, Jalgaon.

***Corresponding Author: Ms. Manasi S. Wagh**

Department of Pharmaceutical Quality Assurance, Kai. Yashodabai Dagadu Saraf Charitable Trust's College of Pharmacy, Sakegaon, Jalgaon, India.

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ABSTRACT

A simple, precise, accurate and stability-indicating RP-HPLC method was developed and validated for the estimation of Abrocitinib in bulk and pharmaceutical dosage form. The analysis of Abrocitinib in methanol using UV spectrophotometric analysis yielded a well-defined absorption maximum (λ max) which showed that it could be detected analytically. The sharp and clear peak indicated the purity of the analyte and the absence of interference. The chromatographic conditions were optimized based on systematic trials on varying mobile phase compositions. Trial-07 was found to be optimized performing trial in terms of chromatographic performance as it had a retention time of about 4.62 minutes, good peak symmetry (0.92), and acceptable theoretical plate count, allowing good separation and reproducibility. The method had a high linearity in 10-50 µg/mL concentration range with a regression equation of Y = 98.85x + 7.317 and correlation coefficient (R 2) of 0.999 indicating a good linear relationship between concentration and peak area. Recovery studies at 80%, 100% and 120% levels confirmed the accuracy of the method with mean recoveries of 99.74%, 100.69% and 99.34% at each level. The values of the %RSD were below 0.5% indicating good accuracy and no excipients interference. Intraday and interday precision studies demonstrated a very low level of the values of %RSD, below 2%, indicating outstanding repeatability and reproducibility of the method. Repeatability studies also showed that there were stable peak areas with a percentage relative standard deviation of 0.08%. The sensitivity of the procedure was confirmed with low LOD (0.1275 µg/mL) and LOQ (0.3864 µg/mL) implying that the procedure can identify and measure the drug at very low levels. Ruggedness and robustness experiments indicated that small intentional variations in analytical conditions like mobile phase composition and detection wavelength did not have any significant impact on the chromatographic performance. The values of the %RSD were in the satisfactory range that validated the applicability of the method in different conditions. Marketed formulation assay revealed a close to 100% (mean 100.62%) of label claim, which demonstrated that the method could be used as a routine analysis of quality control. Studies on forced degradation at acidic, alkaline, oxidative, and neutral conditions revealed that Abrocitinib is highly vulnerable to oxidative degradation followed by alkaline degradation, and acidic degradation and stable in neutral conditions. Photolytic studies confirmed that the drug is stable under UV exposure. The degradation products were well resolved from the main drug peak, confirming the stability indicating nature of the method.

KEYWORDS: RP HPLC, Analytical method development, Abrocitinib.

INTRODUCTION

The pharmacological treatment of moderate-to-severe atopic dermatitis (AD), a chronic inflammatory skin disease afflicting millions of people globally, with its intensive pruritus, eczematous lesions, and altered skin barrier functioning, abrocitinib, Cibinqo, the drug developed that has become the significant advancement in as an oral therapy with a single daily dose of 100 mg (a low dose), abrocitinib was the first JAK inhibitor to receive a favourable benefit-risk profile based on extensive clinical trials, making it an easy-to-use alternative to inject.

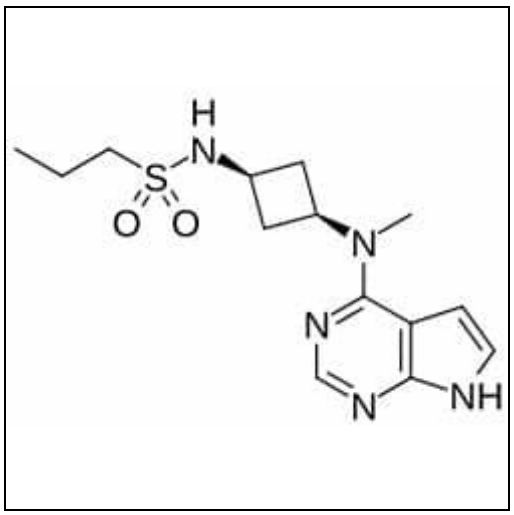

Fig. 1: Strcture of Abrocitinib.

It has chemical name 1-[1-(1,1-Dimethylpropyl)-3-ethyl-1H-pyrazol-5-yl]-3-[[[3-(4-methylpiperazin-1-yl)phenyl]methyl]amino]propan-1-one and molecular formula $C_{26}H_{27}N_5O_2$. The molecular weight of abrocitinib is 423.53 g/mol. It is Practically insoluble in water; soluble in organic solvents. The mechanisam of action Abrocitinib is a selective, reversible JAK1 inhibitor that blocks the ATP-binding site of JAK1, reducing signaling through inflammatory cytokine pathways involved in atopic dermatitis. This lowers downstream immune activation and helps reduce skin inflammation and itch.[1-5]

MATERIALS AND METHODS

Chemicals

The pure drug samples of Abrocitinib were received as a gift sample from Swapnroop Drugs and Pharmaceuticals, Chatrapati Sambhajinagar, India The marketed tablet formulation Cibinqo 100 mg Tablets was purchased from local medical. HPLC grade methanol, acetic acid, and purified water were purchased from Merck India Ltd. All solvents and reagents were filtered through a 0.45 µm membrane filter and then degassed prior of using for removal of any particulate matter and dissolved gases. The chemicals and reagents used in the study were of analytical reagent grade or HPLC grade for ensuring accuracy, reliability, and reproducibility of the analytical method.

Instrumentation and analytical conditions: The chromatographic analysis was carried out using an Agilent 1100 HPLC system equipped with a diode array detector (DAD, G1314B) and ChemStation software for data acquisition and processing.The stationary phase RP-C18 (Agilent) column 4.6 × 250 mm with pack particle size 2.5 micro meter packing stationary phase. Mobile phase consist of Methanol and 0.1 % Formic acid buffer in 69:31 % w/v.

The system was capable of operating at a maximum pressure of 400 bar with a discharge flow rate range of 0.001 to 5 mL/min. The pressure display accuracy was maintained at ±5%, and the system could accommodate up to four mobile phases with a mixing ratio range of 0 to 100%. The pump unit was a high-performance reciprocating pump (HP-1100), which ensured precise and consistent solvent delivery during analysis.

Wavelength selection

The prepared working solution of ACB (10 µg/mL) in methanol was scanned in the wavelength range 200–400 nm using methanol as blank. The wavelength showing maximum absorbance (λ max) for ACB was recorded.

Preparation of standard solution

Precisely weighed quantity of 10 mg of ACB was transferred into clean, dry 100 mL volumetric flask. 20–30 mL of methanol was added to the flask and the content was subjected for sonication for 10 minutes to ensure complete dissolution. After sonication, the volume in each flask was made up to the mark of 100 mL with methanol to obtain stock solution having 100 µg/mL concentrations.

Preparation of Sample solution

From the above stock solution 10 mL of the stock solution (100 µg/mL) was pipetted into a 100 mL volumetric flask and the volume was made up with methanol to obtain a solution of 10 µg/mL.

RESULT AND DISCUSSION

Method optimization

The selection of the mobile phase was based upon assessment of various combinations of methanol and 0.1% formic acid. The ratios ranging from 90:10 to 59:44 (v/v) were tested under the same chromatographic conditions. Each composition was freshly prepared, degassed, filtered and injected with standard solutions in order to determine retention time, peak shape and system suitability. The chromatographic responses for each trial were recorded and evaluated.

Table no 1: Method Development Trials for Abrocitinib.

Trial No.	Mobile Phase Composition (v/v)	Flow Rate (mL/min)	Injection Volume
Trial 01	MeOH 90% + 0.1% Formic Acid 10%	0.7	20 µL
Trial 02	MeOH 80% + 0.1% Formic Acid 20%	0.7	20 µL
Trial 03	MeOH 75% + 0.1% Formic Acid 25%	0.7	20 µL
Trial 04	MeOH 65% + 0.1% Formic Acid 35%	0.7	20 µL
Trial 05	MeOH 59% + 0.1% Formic Acid 44%	0.7	20 µL
Trial 06	MeOH 59% + 0.1% Formic Acid 41%	0.7	20 µL
Trial 07	MeOH 69% + 0.1% Formic Acid 31%	0.7	20 µL

Final chromatographic condition: Trial 07 showed an optimal chromatographic profile with a retention time of 4.621 minutes, acceptable plate count (4591), good peak symmetry (0.92) and sharp and well defined peak. On the basis of these observations, Trial 07 was chosen as the best mobile phase condition, which yielded a good retention time, even peak shape, and stable chromatographic behavior.

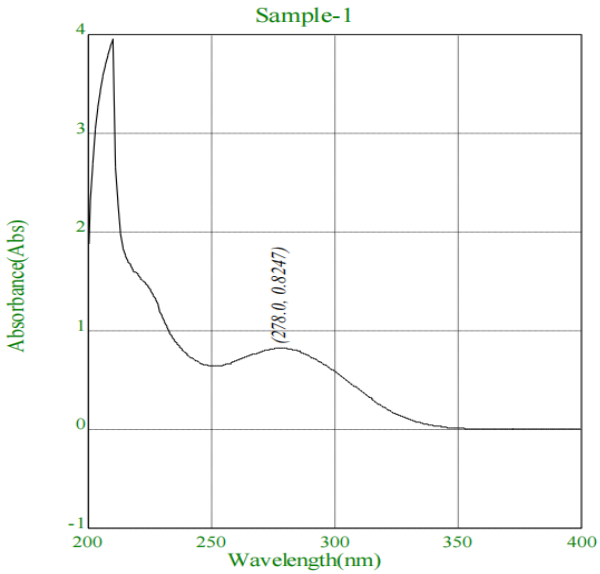

Fig. 3: UV absorption spectrum of Abrocitinib (10 µg/mL) in methanol.

Linearity: The linearity of ACB was evaluated over the concentration range of 10–50 µg/mL. The outcome illustrated a linear relationship between the concentration and the response as the peak area was proportional to the concentration The peak area increased from approximately 992.79 mAUs at 10 µg/mL to 4945.36 mAU·s at 50 µg/Ml recorded between 4.30 to 4.52 in all the concentrations thus, it was in the stable chromatographic conditions. The regression equation that was obtained using the calibration curve was Y=98.85x+7.317, and the correlation coefficient (R2) was 0.999.

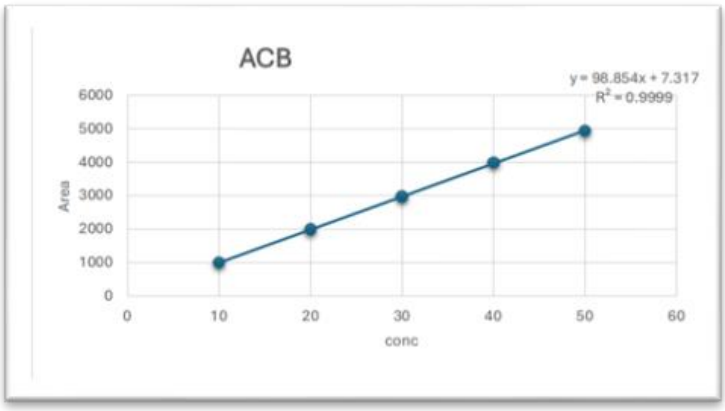

Table no 2: Linearity Parameters of Abrocitinib.

Sr no	Concentration	Peak area	Height	Theorotical plates
1	10 µg	997.76154	86.68520	4291
2	20 µg	1986.15063	175.78305	4165
3	30 µg	2961.27002	271.91061	4269
4	40 µg	3983.28076	425.16803	4253
5	50 µg	4931.07764	444.34628	4438

Accuracy

The accuracy of the method was assessed by recovery studies at three levels of 80%, 100% and 120%. The average recoveries were found to be 99.74%, 100.69%, and 99.34%, respectively, and which fall within acceptable pharmacopeial range of 98-102%. These results show that the method is highly accurate and capable to give actual results without excipients interference. The values of the %RSD were observed to be less than 0.5%, which indicated low variability and high accuracy in the course of recovery studies. The results confirm that the technique has a high trueness and reliability to do quantitative estimation.

Table no. 3: Accuracy parameters of Abrocitinib.

Sample Label	Area	Height	Ret Time	Symateery	Plate
80	1786.23511	**160.88**	**4.495**	**0.90**	**4251**
100	1991.38879	**179.37**	**4.539**	**0.92**	**4363**
120	2178.27612	**194.37**	**4.476**	**0.92**	**4056**
Sample Label	Conc of API Added	Amount Recovered	Mean	S D	% RSD
80	**8**	**8.00**	**99.74**	**0.30**	**0.30**
100	10	**10.07**	100.69	**0.04**	**0.03**
120	12	**11.96**	**99.34**	**0.48**	**0.48**

PRECISION

Precision is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings.

The intraday precision was determined by using three levels of concentration 20, 30 and 40 µg/mL) within a same day. The retention times were consistent and ranged between 4.49 and 4.52 minutes, this reflected stable chromatographic conditions. The %RSD values for peak area were found to be 0.16%, 0.11%, and 0.04% for the respective concentrations, demonstrating excellent repeatability. The observed percentage value was between 99.76% and 100.74% which showed accurate quantification. These findings reveal that the method developed is highly precise in the same-day conditions of analysis.

Table no 4: Intraday Precision Parameters Abrocitinib.

Sr no	Concentration	Mean Peak area	% Amount Found	S D	%RSD
1	**20**	1979.54	99.76	3.26	0.16
2	**30**	2965.86	99.77	3.41	0.11
3	**40**	3990.70	100.74	1.73	0.4

Interday precision was assessed across different days to find out how reproducible the method is. Retention times were constant with a range of 4.32 to 4.40 minutes indicating the method to be robust over the days. The values of %RSD were between 0.05% to 0.68% which is acceptable. The percentage amount obtained was between 99.63% and 100.86%, which validated the uniformity in the performance of analysis. These findings indicate that the technique is very reproducible and suitable to be used to conduct regular analysis across various days.

Table no. 5: Intraday Precision Parameters Abrocitinib.

Sr no	Concentration	Mean Peak area	% Amount Found	S D	%RSD
1	**20**	1979.54	99.63	6.19	0.31
2	**30**	2965.86	100.07	20.24	0.68
3	**40**	3990.70	100.86	2.12	0.05

LOD & LOQ: The standard deviation of the response and slope of the calibration curve was used to calculate the LOD and LOQ according to the ICH guidelines. LOD was observed to be 0.1275µg/ml,
Whereas the LOQ was 0.3864 µg/ml. The values of LOD and LOQ are low and these values
LOD = 3.3 × 3.82/98.85

LOD =12.606/98.85
=0.1275

LOQ =10 × 3.82/98.85

LOQ =38.20/98.85
= **0.3865**

Repeatability
Repeatability was studied by repeated injections of the same concentration under identical conditions. The chromatographic response was very reproducible with minute changes in the amount of the peaks and the retention time. The average values of the mean % RSD of peak response were very low, showing that the method is very repeatable.

Repeatability was tested with replicate samples injected at the concentration of 40 µg/ mL. Retention time was constant (4.46–4.48 minutes), and the peak areas were very consistent. The peak area %RSD was calculated to be 0.08% which implies that the method is highly reproducible. The percentage value obtained was 100.35%, which are accurate and consistent inrepeated measurements.

Table no. 6: Repeatability Parameters of Abrocitinib.

Injection	Ret time	Area(mAU·s)	Plates
1 40	4.463	3972.92041	4378
2 40	4.479	3977.23535	4394

Ruggedness and Robustness
The method ruggedness was measured by analysing the method in different conditions. The retention times showed negligible variation (4.42–4.43 minutes), indicating stability of the method under different operational conditions. The %RSD was determined to be 0.173% and the label claim was around 100.79%, indicating that the slight changes in the conditions of the analysis would not have a significant impact on the outcome. These results affirm the ruggedness of the method.

The robustness was tested by subjecting small deliberate changes in mobile phase composition and detection wavelength. The results revealed that changes in mobile phase composition (68:32 and 70:30 MeOH:Water) resulted in the production of less than 0.02% %RSD, which means that there was no significant impact on the performance of the chromatography.

Similarly, the change in detection wavelength (277 nm and 279 nm) produced a mean percentage of %RSD of about 0.11% and this proved that there were very few effects on the analytical results. Theoretical counts of plates and peak symmetry were kept at acceptable levels given all the conditions. These observations explain that the procedure is exceedingly strong and dependable when there are minor changes in the analysis parameters.

Table no 7: Robustness Parameters Abrocitinib.

Condition Change	Retention Time	Area	Plate
Mobile phase 68:32	4.568	4983.81250	4152
Mobile phase 71:30	4.538	4916.33984	4633
Wavelength 277 nm	4.517	4927.39600	4339
Wavelength 279 nm	4.517	4765.29932	4252

Assay of Abrocitinib
The developed method was used to assay ACB in the marketed tablet formulation (CIBINQO 100 mg) with the concentration of 40µg/ml. The retention time was stable (4.51–4.52 minutes) and the peaks were sharp and symmetrical. The % label claim values were found to be 100.68% and 100.56%, with a mean value of 100.62%. The method was very precise and accurate as the %RSD was 0.087%. The obtained findings support the claim that the method developed is applicable to the routine quality control analysis of ACB in pharmaceutical products.

Table no 8: Assay Parameters Abrocitinib.

Formulation	Area	Amount Found (µg/mL)	% Label Claim (mg)
Tablet A	3988.33	40.27	100.68
Tablet B	3983.44	40.22	100.56
Mean	3985.88	40.25	100.62
S D	3.45	0.035	0.087
% R S D	0.087	0.087	0.087

Forced Degradation Studies
Forced degradation studies were carried out under acidic, basic, neutral, and oxidative conditions to evaluate the stability-indicating nature of the method. The moderate degradation was observed under acidic condition (0.1 N HCl, 2 hours) where main peak was retained and no

significant interference was noted. Alkaline conditions (0.1 N NaOH, 2 hours) led to a slight degradation and the appearance of degradation products. There was no significant degradation in neutral conditions implying that the drug is stable in aqueous conditions. The oxidative degradation (3% H_2O_2, 2 hours) caused high degradation with the formation of several degradation products, showing that the drug is highly susceptible to oxidative stress.

Table no 9: Forced Degradation Studies Abrocitinib.

Degradation Condition	Area of Standard	Area of Degraded Sample	% Remaining	Actual % Degradation
Acid Degradation	2962.79	2552.75	86.16	13.84
Basic Degradation	2962.79	2523.10	85.16	14.84
H_2O_2 Degradation	2962.79	2430.43	82.03	17.97
Neutral Degradation	2962.79	2950.11	99.57	0.43

CONCLUSION

A simple, sensitive, precise, accurate and stability-indicating RP-HPLC method has been successfully developed and validated for the estimation of Abrocitinib in bulk and pharmaceutical dosage forms. The method developed meets the criteria of ICH validation, and exhibits high linearity, accuracy, precision, robustness, ruggedness, and sensitivity. The optimal chromatographic conditions achieved effective separation of the drug and the degradation products. The method proved to be stability-indicating through forced degradation studies, where all degradation products were effectively separated without interference with the main peak. The developed RP-HPLC method can be effectively used in pharmaceutical industries and research laboratories due to its simplicity, reproducibility, and reliability allowing quality control analysis, pharmaceutical formulation assay, stability analysis, and regulatory us.

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