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DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR THE ESTIMATION OF ABROCITINIB IN TABLET DOSAGE FORM

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ABSTRACT

A new, selective, reliable analytical RP-HPLC method for estimating Abrocitinib in solid dosage form was developed and validated. The chromatographic analysis was carried out on a reverse phase analytical column of Inertsil C-18 (250 mm x 4.6 mm, 5µm) column with a mobile phase of Water: Acetonitrile in a 60:40 v/v ratio at a flow rate of 1 ml/min. At a wavelength of 287 nm, the desired analyte was identified without any interference. The retention time of the drug was discovered to be 5.2 minutes. The graphical method produced linear responses with a regression coefficient of 0.9997 for the concentration range of 10-130 µg/ml of. The LOD and LOQ values were 5 and 10 µg/ml, respectively. These results obtained, showed a good agreement with the declared content in that of the formulation. Therefore, the proposed method is rapid, accurate, and validated for the quantification of abrocitinib in solid dosage form.

KEYWORDS: RP-HPLC, Method development, Validation, Abrocitinib.

INTRODUCTION

Atopic dermatitis (AD) or eczema is a chronic inflammatory condition characterized by common symptoms like red to brownish-grey coloured patches, itching, which may be severe, especially at night. (National Institute of Arthritis and Musculoskeletal and Skin Diseases, n.d.) It also consists of small, raised bumps, which may leak fluid and crust over

when scratched. Despite the recent advancement in treatments, topical therapies have limited efficacy in moderate-to-severe disease because of its tolerability issues. Severe chronic itch also impacts sleep and productivity. The U.S. Food and Drug Administration (FDA) approved Pfizer's CIBINQO Abrocitinib in January 2022, as a once-daily oral treatment with proven efficacy to manage and control symptoms for atopic dermatitis in adolescents and adults. (FDA Approves Pfizer's Supplemental New Drug Application for CIBINQO® (abrocitinib), 2023).

Chemically, Abrocitinib is N-((1s,3s)-3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)cyclobutyl)propane-1-sulfonamide. (Fisher Scientific, n.d.) The molecular formula is $C_{14}H_{21}N_5O_2S$ and the molecular weight is 323.41g/mol. (Medchem Express.com, n.d.) The excipients used for the tablet is lactose monohydrate. (Cibinqo, INN-abrocitinib, n.d.) Appearance of the drug in powdered form is white to pale colour. The polymorphism of this drug was found to be in crystalline anhydrous form. (Pfizer Medical Information, n.d.) When pH values are less than 4, the drug possesses the characteristics of a highly soluble compound that dissolves rapidly. Whereas when the pH values are greater than 4, the drug behaves as a low soluble compound and hence dissolves slowly. According to the solubility data in IP Volume 1, the drug falls in the category of ‘very slightly soluble’ and thus, is non-polar in nature. (Table 2.4.26 Solubility, 2022) This drug is non-hygroscopic with a melting point of ~189℃, pKa and log P values are 5.3 and 1.66 respectively. (Drug Bank, n.d.).

Abrocitinib is an oral, once-daily, Janus kinase 1 (JAK1) inhibitor, for the treatment of refractory, moderate-to-severe AD. (Janus Kinase Inhibitors for the Treatment of Atopic Dermatitis: Focus on Abrocitinib, Baricitinib, and Upadacitinib, 2021). Janus kinases are intracellular enzymes involved in transduction pathways that regulate hematopoiesis and immune cell function. (Highlights of Prescribing Information, n.d.) The chemical structure of Abrocitinib is shown in figure 1 respectively. During the literature survey, no developed HPLC method was found for Abrocitinib. Hence this study was done to develop a reliable, accurate and precise method for the analysis of Abrocitinib in tablet dosage form.

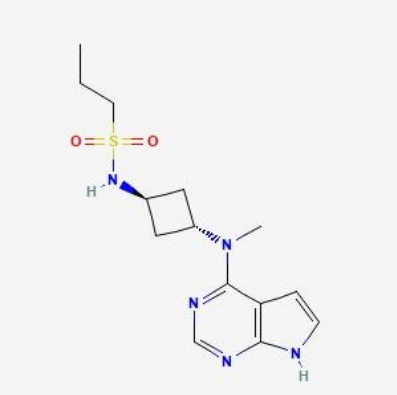

Fig.1: Structure of Abrocitinib (PubChem, n.d.).

MATERIALS AND METHODS

Instrumentation

The analysis of Abrocitinib was performed on WATERS 2695 Alliance HPLC system which was apparelled with WATERS 2996 Photo Diode Array Detector. "Empower 3" software was used for data processing and evaluation. For all the spectrophotometric measurements, Lab India UV/VIS Spectrophotometer having UV Vis Analyst software version **[**6.0.4.0738**]** was used. Sartorius Analytical Balance was used for all the weighing.

Chemicals and Reagents

Abrocitinib Reference Standard having potency of 99% was obtained from Central Drugs Testing Laboratory, Mumbai. Abrocitinib tablets with the brand name Cibinqo® containing 200 mg Abrocitinib was received as a testing sample from Pfizer. Acetonitrile (AR grade) was used.

Determination of wavelength of maximum absorbance- For determining absorbance, 10 mg of Abrocitinib standard was weighed accurately and transferred to 100 mL volumetric flask and volume was made up to the mark using diluent (100 µg/mL). This solution was scanned in the range of 400.0 nm to 200.0 nm using UV/Visible spectrophotometer keeping methanol as blank. Abrocitinib showed maximum absorbance at 287 nm. As a result, the same wavelength was chosen for Abrocitinib analysis.

Preparation of Mobile phase

A mixture of Water and Acetonitrile in the ratio of 60:40 (v/v) was used as mobile phase for the study.

Preparation of Standard Solution

A 100 µg/mL stock solution of Abrocitinib was prepared. For preparation of the working standard solution, 10 mg of Abrocitinib standard was weighed accurately and added to the volumetric flask with some amount of diluent to dissolve the standard. The diluent used was the same as mobile phase. While dissolving, all fine particles of the standard were completely dissolved before proceeding with its analysis. After dissolving, the volume was made up using diluent so as to prepare a 100 ppm concentrated stock solution. This solution was used as the standard solution for analysis.

Preparation of Sample Solution

In total, 5 tablets of Abrocitinib were weighed and the average weight was determined. The tablets were crushed thoroughly until fine particles were observed. An accurately weighed amount of powder equivalent to 10 mg of Abrocitinib was transferred to six different 100 mL volumetric flask. This was followed by the addition of some amount of diluent to dissolve the sample. The solution was then sonicated for at least 25 minutes. Further, the volume was made up to the mark using diluent. This sample solution was filtered through a 0.45 membrane filter.

Method development and optimization

Several literatures were obtained about the physical and chemical properties of Abrocitinib. Molecular, structural and solubility data revealed that Abrocitinib is a polar drug, Initial trials on HPLC were carried out on Inertsil C18 [ODS-3V 5µm (4.6 mm × 250 mm)] column by considering the chemical nature of the molecule.

Different trials were done by using mobile phase systems in different ratios to obtain the separation of Abrocitinib with Trifluoroacetic acid 0.1 % (pH3) 70:30 (v/v), Triethylamine 70:30 (v/v), Formic acid 0.1%. These three mentioned mobile phases gave poor peak shape and bad symmetry. Also, very short retention times and peaks with lower resolution was observed. Variations were made in mobile phase proportion, injection volume, and concentration of the standard solution. But none of the conditions gave acceptable S.S.T. parameters. Hence further trials were done by using Water: Acetonitrile 65:35 (v/v). This trial was done on Inertsil C18 [ODS-3V 5µm (4.6 mm × 250 mm)] column. Various trials were done with this mobile phase in different ratios, injection volume and concentration of the standard solutions of the analyte. Chromatograms with acceptable peak shape and SST parameters were obtained on this column. Eventually, good peak shape, resolution and

acceptable system suitability parameters were found with the mobile phase composition of Water: Acetonitrile 60:40 (v/v) and Inertsil C18 [ODS-3V 5µm (4.6 mm × 250 mm)].

The desired peak shape, size and resolution were obtained with the following chromatographic conditions:

Column: Inertsil C18 [ODS-3V 5µm (4.6 mm × 250 mm)]

Flow rate: 1 mL/min.

Mobile phase and diluent: Water: Acetonitrile 60:40 (v/v)

Programming: Isocratic

Wavelength: 287 nm.

Run time: 10 min.

Injection volume: 10 µL

Temperature: 40℃

Method Validation Studies

The developed RP-HPLC method for Abrocitinib was validated for parameters such as specificity, linearity, precision, accuracy, LOD, LOQ, and robustness according to ICH guidelines.

Specificity- Specificity refers to the ability of the method to detect only the target analyte and its non- interference with other components in the sample. For this, 10 µL solution of blank, standard and sample solution were injected into the HPLC system separately and the chromatograms are shown in Figures 2-4. There are no co-eluting peaks observed at the retention time of Abrocitinib which indicates that the peak of the analyte was pure. Therefore, the specificity of the method is confirmed.

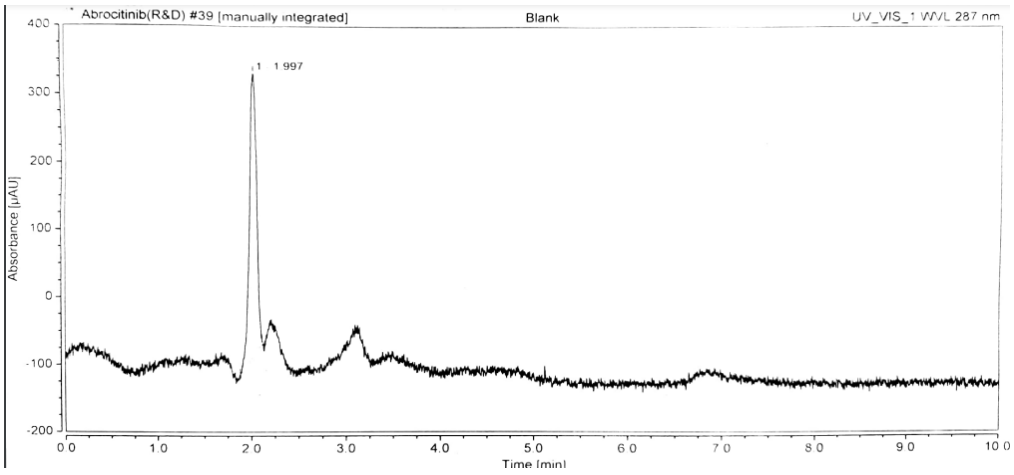

Fig. 2: Chromatogram of Blank.

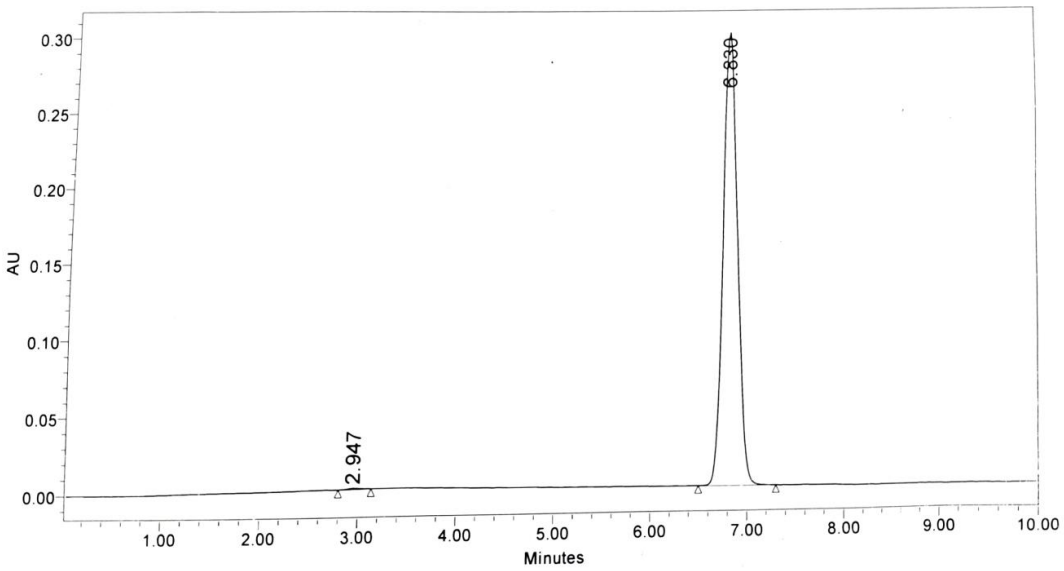

Fig. 3: Chromatogram of Standard.

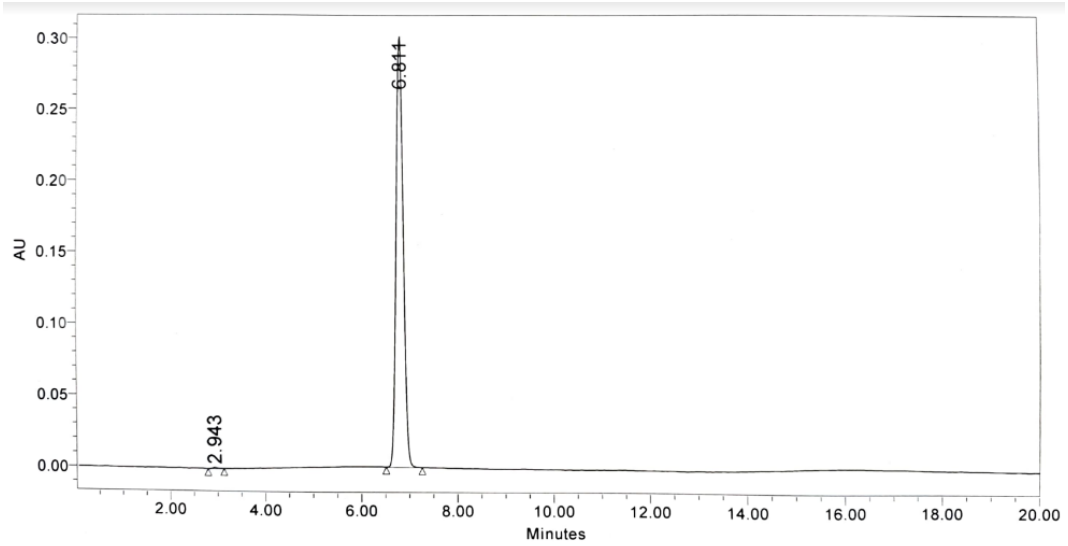

Fig. 4: Chromatogram of Sample.

System Suitability

System suitability parameter is for verifying the method developed as per its purpose suitability. Therefore, parameters were analysed to check the system performance by injecting mixed standard preparation (six replicates) into the HPLC system. The chromatograms were recorded to evaluate S.S.T. parameters like % RSD of R.T., resolution, tailing factor and theoretical plates.

Table 1: System suitability parameters for Abrocitinib.

Injection no	Area of drug	Retention time	Tailing factor	Theoretical Plates
1	6221031	6.81	1.23	7048
2	6253956	6.83	1.23	7067
3	6329696	6.83	1.21	7039
4	6233071	6.79	1.22	7010
5	6291200	6.85	1.20	7007
6	6121924	6.86	1.20	7040
Mean	6241813	6.828	1.215	7035.167
SD	70981.17541	0.02563	0.013784	22.9905
% RSD	1.137	0.37	1.134	0.326
Limit	NMT 2.0%	NMT 1.0%	NMT 2.0%	NLT 2%

Linearity

Linearity is carried out, so as to ensure that the series depicts area as a linear corresponding feature to that of its concentration. The linearity range for this method was studied by preparing standard solutions of Abrocitinib in different concentrations. A standard stock solution of Abrocitinib was prepared which was diluted with mobile phase so as to prepare concentrations of Abrocitinib within the range of 10-130 µg/mL. Three injections from each concentration were prepared and analysed under the same conditions. The mean peak area of Abrocitinib was plotted against corresponding concentrations to obtain the calibration results. The results of linearity studies showed a linear relationship over the concentration range of 10-130 µg/mL for Abrocitinib. From the regression analysis, the linear equation obtained was: $y = 973.47x + 2269.7$, and the correlation coefficient (R^2) value was found to be 0.9997 indicating a linear relationship between the concentration of analyte and area under the peak.

Table 2: Linearity data for Abrocitinib.

Linearity level	Concentration	Peak area
1	10	11470.71
2	25	24130.99
3	50	52895.91
4	75	71961.51
5	100	106111.8
6	120	122967
7	130	127456.3

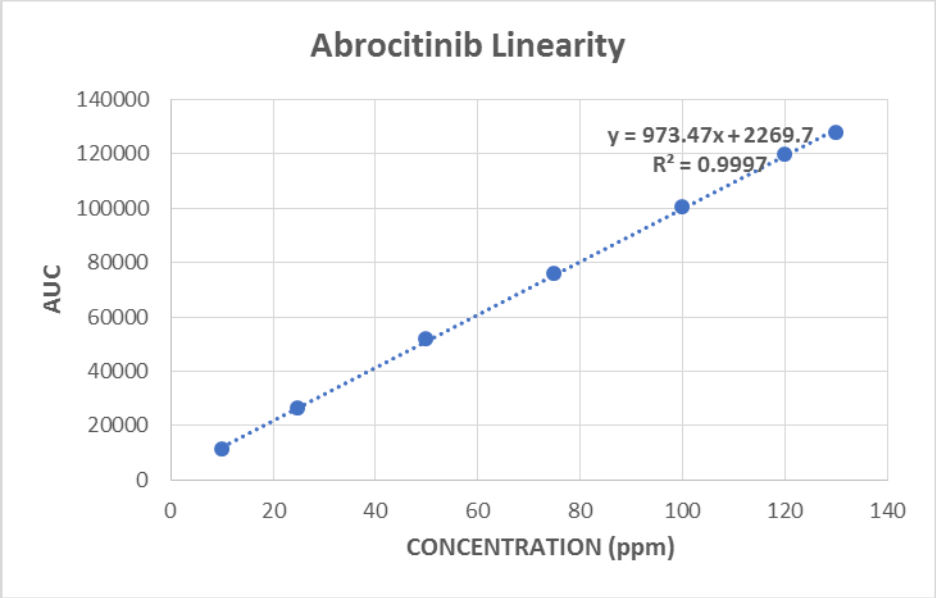

Fig. 5: Linearity Graph.

Sensitivity

Sensitivity refers to the ability of the method and system to detect the lowest concentrations possible of an analyte. LOD calculation based on the standard deviation of the response and the slope of a calibration curve is done using the following formula:

$$LOD = 3.3^* \propto /S.$$

Where ∝ is the standard deviation of the response and S is the slope of the calibration curve.

The Limit Of Quantification (LOQ) is calculated similarly as the LOD using the formula:

$LOQ = 10^* \propto /S.$

The theoretically obtained LOD and LOQ value of Abrocitinib was calculated to be **5 µg/mL** and **10 µg/mL** respectively.

Table 3: Experimentally derived sensitivity data of Abrocitinib.

Molecule	LOD	LOQ
Abrocitinib	5 µg/ml	10 µg/ml

Accuracy

Accuracy is a parameter that determines the trueness of an experimental method. The strength of the tablet used for this parameter The accuracy for the developed method was determined by recovery studies at three concentration levels (110%, 120%, and 130%) by standard addition method. Three samples of each concentration were injected. For each replicate sample, the percentage recovery of added Abrocitinib and R.S.D were calculated. The mean % recovery was 99.6654.83%.

Table 4: Accuracy studies of Abrocitinib.

% level	Standard spiked with sample (mg)	Amount recovered (mg)	% Amount recovered	% Recovery	Mean Recovery
100	0	196.686	98.343	98.343	99.6654 %
110	20	219.8812	109.9406	99.9464	
120	40	240.0724	120.0362	100.03	
130	60	260.886	130.4448	100.3423	

Precision

It refers to repeatability and reproducibility of the method. Precision is evaluated by analysing replicate samples and calculating the relative standard deviations. The method precision (repeatability) was determined by several measurements of standard. For System precision, six measurements of the standard solution at the 100% concentration levels was determined on the same day. Method precision was established by six assay determinations of the sample solution at the 100% concentration levels on two different days. The %RSD and results obtained were calculated to evaluate repeatability of the results according to which all the values were found to be within limit.

Table 5: Precision data as Repeatability.

Parameters	AUC for standard	AUC for sample
1	6304355	6155683
2	6227820	6121742
3	6178930	6156422
4	6204149	6250017
5	6254783	6208377
Mean	6234007	6178448.2
SD	48331.50	56536.151
% RSD	0.78	0.92
Limit	NMT 2%	NMT 2%

Robustness

Robustness of an analytical procedure is its capacity to obtain similar and acceptable results on introducing small but deliberate variations through procedural parameters. Robustness of the proposed method was analysed by changing flow rate (± 2 mL), Mobile Phase composition (± 2%) and wavelength (± 2 nm). Under such different chromatographic conditions, three sample solutions of Abrocitinib were prepared and injected in triplicates along with six replicate injections of the working standard solution. Mean, SD, and % RSD of % estimation were calculated and reported. The results of robustness studies showed that a minor change in the method condition, such as phase ratio, flow rate and wavelength did not

show any significant deviation in the results. The recovery and %RSD were within the acceptable limits.

Table 6: Robustness studies of Abrocitinib.

Parameter	Change in parameter	% estimation	Mean	SD	%RSD	Limit
Wavelength (± 2nm)	285	99.45	100.1554	1.344971	1.34	NMT 2.0%
	287	101.7064				
	289	99.31				
Flow rate (± 0.2 ml)	0.8 ml	99.48	100.5275	1.061618	1.05	
	1.0 ml	101.6027				
	1.2 ml	100.50				
Mobile Phase composition (± 2%)	65:35	100.27	100.5303	1.054875	1.04	
	60:40	101.6910				
	55:45	99.63				
Temperature	38	99.45	100.1554	1.344971	0.0134	
	40	101.7064				
	42	99.31				

Assay

The assay results obtained shows high percentage recoveries and low SD values which confirms that the method is suitable for routine analysis of Abrocitinib in its tablet dosage form.

Table 7: Assay results for Abrocitinib.

Sr. no	Weight of Standard (mg)	Sample weight (equivalent to 10 mg)	Assay (%)
1	10.36	34.08	101.10
2		33.81	101.35
3		34.06	101.17
4		34.52	101.34
5		34.13	101.82
6		34.233	101.51
		Mean	101.38
		S.D.	0.25903
		R.S.D (%)	0.256%

RESULTS AND DISCUSSIONS

A novel, precise and accurate RP-HPLC method for determining Abrocitinib in tablet dosage form has been devised. In reference to the chemical composition of Abrocitinib, the chromatographic conditions were optimized. Six duplicates of the standard solution

containing a working concentration of 100µg/ml Abrocitinib were used to evaluate System Suitability characteristics. The percentage R.S.D of factors including area, retention time, tailing factor and resolution were calculated. All parameters for this approach were confirmed to be within acceptable limits. Table 1 summarizes the findings of system suitability parameter. Figures 2,3, and 4 show the chromatograms of the blank, standard and sample respectively. The linearity studies revealed a linear detector response with a correlation coefficient of 0.9997 for the concentration range of 5-150 µg/ml. As illustrated, in Fig.5, the regression equation found was y = 973.47x + 2269.7. Table 2 displays the linearity data. As shown in table 3, the LOD and LOQ values for Abrocitinib were 5 µg/ml and 10 µg/ml respectively. As a result, the approach was found to be sensitive over a wide range. The method was determined to be accurate, with a mean percent recovery of 99.6654% for Abrocitinib sample solutions. For precision parameters to be validated, Abrocitinib standard solutions were determined to be within limits, indicating that the procedure was exact. The intermediate precision was obtained by analysing Abrocitinib tablets using the developed method. As indicated in Table 5, the assay's % RSD values were confirmed to be within acceptable criteria. The method was sufficiently robust to account for variations in parameters such as mobile phase ratio, flow rate, wavelength and temperature. The %RSD of the test with changes in method parameters was less than 2.0 %, and the results were unaffected. Table 6. summarizes the findings. The assay results shown in Table 7 demonstrated high percent recoveries and low SD values, confirming that the procedure as well as the method is effective.

Abbreviations

RP-HPLC: Reversed Phase High Performance Liquid Chromatography; LOD: Limit of Detection; LOQ: Limit of Quantification; ICH: International Council for Harmonization; AR: Analytical reagent; UV-VIS: Ultraviolet-visible spectrophotometry; RT: Retention Time; NMT: Not More Than; SD: Standard deviation; %RSD: Percentage Relative Standard Deviation.

CONCLUSION

Thus, the RP-HPLC method developed for abrocitinib is in adherence to all the parameters such as linearity, accuracy, precision, reproducibility and specificity as apparent from the validation results. All of the checked parameters are in accordance to the ICH guidelines. Estimation of regression values, R.S.D. (%) and standard deviation gives a multi-faceted

approach to this developed method, thus making it very adaptable in routine usage. Hence the developed RP-HPLC technique can be utilized for the standard analysis of abrocitinib drug in its solid dosage form.

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