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METHOD DEVELOPMENT AND VALIDATION OF BARICITINIB IN BULK AND PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC

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ABSTRACT
A simple rapid, precise, sensitive reproducible reverse phase high performance liquid chromatography (RP-HPLC) has been developed for the quantitative analysis of baricitinib in pharmaceutical dosage form. Chromatography separation of Baricitinib was achieved on waters alliance E2695 by using Symmetry shield RP18. (4.6x150mm, 3.5µm) The mobile phase Containing Acetonitrile and 2.5gK_2HPO_4+3.2gKH_2PO_4(adjust pH 3.0 with OPA) in the ratio of 40:60. The flow rate was 1ml/min, detection was Carried out by absorption at 257nm using a photo diode array detector at ambient temperature. The number of theoretical plates and tailing factor for baricitinib was not less than 2000 and should not be more than 2 respectively. The percentage of relative standard deviation of peak areas of all measurements is always less than 2. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate, and robust method for quantitative analysis of Baricitinib.

Keywords: RP-HPLC, Baricitinib, development, validation.

INTRODUCTION:

Chromatography is the most powerful analytical technique available to determine the individual components present in a mixture quantitatively [1,2]. . High-performance liquid chromatography (HPLC) is a chromatographic technique that can separate a mixture of compounds and is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of mixture [3] . Reversed phase chromatography has found both analytical and preparative applications in the area of biochemical separation and purification. Molecules that possess some degree of hydrophobic character, such as proteins, peptides and nucleic acids, can be separated by reversed phase chromatography with excellent recovery and resolution [4] . Now a day reversed-phase chromatography is the most commonly used separation technique in HPLC due to its broad application range. It is estimated that over 65% (possibly up to 90%) of all HPLC separations are carried out in the reversed-phase mode. The reasons for this include the simplicity, versatility, and scope of the reversed-phase method as it is able to handle compounds of a diverse polarity and molecular mass [5-8].

High-performance liquid chromatography (HPLC) involves the injection of a small volume of liquid sample into a tube packed with tiny particles (3 to 5 microns (µm) in diameter called the stationary phase) where individual components of the sample are moved down the packed tube with a liquid (mobile phase) forced through the column by high pressure delivered through a pump.

Column packing is used to separate the components from one another. It involves various chemical and/or physical interactions between their molecules and the packing particles. The separated components are then detected at the exit of the column by a detector that measures their amount. Output from this detector is called a "liquid chromatogram."

Baricitinib also known as Olumiant, (3-Azetidineacetonitrile, 1-(ethylsulfonyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]) is used to treat rheumatoid arthritis. It is a type of drug known as a Janus kinase JAK inhibitor. It works by blocking the action of Janus kinase enzymes ,which are involved in the inflammation that causes the symptoms of rheumatoid arthritis.

Baricitinib can relieve the symptoms of pain ,stiffness and swelling in your joints and slow the joint damage that rheumatoid arthritis can cause .most people who benefit from this treatment will notice some improvement with in the first 12weeks of treatment.

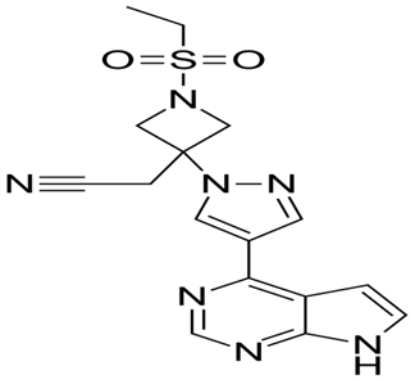

Fig-1: Structure of baricitinib

Materials And Methods:

Alliance waters e 2695 – empower software 2.0 version with Isocratic Pump was used. The PDA Detector with detection wavelength selected was 257 nm. Chromatographic separation was performed using Symmetry shield RP18. (4.6x150mm, 3.5µm) column.

Acetonitrile and methanol were of HPLC grade of Rankem brand, HPLC grade Water (Milli Q)in house production. Analytical grade Dipotassium hydrogen phosphate and potassium dihydrogen phosphate were used.

Diluent: Buffer and Acetonitrile in the ratio of 60:40 used as diluent

Preparation of Standard Solution

Accurately weighed and transferred 20 mg of Baricitinib working standard into a 100ml clean dry volumetric flask added diluent and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution)

Further pipetted 5 ml of the above stock solutions into a 50 ml volumetric flask and diluted up to the mark with diluent. (20ppm of Baricitinib)

Preparation of Sample Solution

76mg of baricitinib was accurately weighed and transferred into 10ml volumetric flask .The components were dissolved in diluent and sonicated for 30minutes to remove air bubbles and the flask was filled with solvent to the required levels .From this 1ml of solution was pipetted out into 10 ml of volumetric flask and dilute up to the mark with diluent. (20ppm of Baricitinib).

Chromatographic procedure:

Chromatographic separations were carried out on Symmetry shield RP18. (4.6x150mm, 3.5µm).A mobile phase used was Buffer and Acetonitrile (60:40) , Wavelength of 257 nm was used for detection, at which the drug showed a good response. Quantitation was performed by using low pressure gradient at 1.0 mL/min flow rate ,run time was 10 minute and column temperature was maintained at ambient temperature. An Injection volume used was 10 µL.

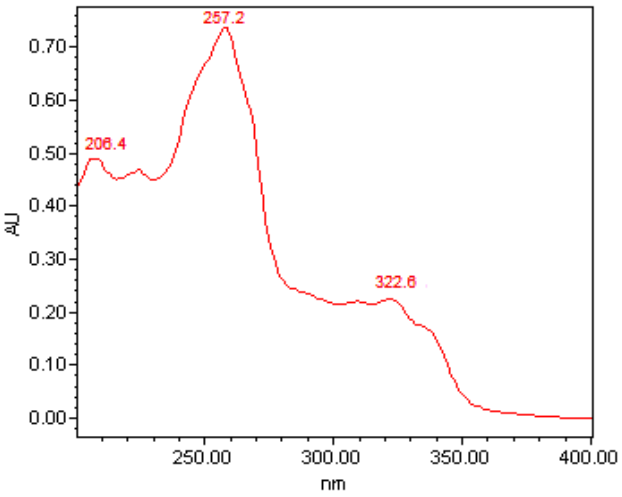

Fig-2: Baricitinib PDA spectra

Method Validation :

The RP-HPLC method was validated according to ICH guidelines for system suitability, specificity, linearity, precision, and robustness.

a)System Suitability or System Precision :

System precision is checked by using standard chemical substances to ensure that the analytical system is working properly. In this peak area and the % of drug of 6 determinations is measured and % of RSD should be calculated. Tailing factor for the peak due to baricitinib in standard solution should not be more than 2. Theoretical plates (plate count) for the baricitinib peak in standard solution should not be less than 2000.Resolution for the baricitinib peak in standard solution should not be less than 2.

b).Specificity(Or)Selectivity:

The specificity of an analytical method is ability to measure specifically the analyte of interest without interference from blank and known impurities. For this purpose, blank chromatography, sample chromatography and standard chromatography of blank shows no response at the retention times of drug which confirm the response of drug was specific.

c).Linearity:

The prepared stock solution is used to make different concentrations of baricitinib required for the experiment.

Levels of preparations :

Table-1:levels of preparation of Baricitinib

Preparation	Volume of standard stock transferred in ml	Volume made up in ml (diluent)	Conc. of Baricitinib in ppm
1	0.2	10	4
2	0.4	10	8
3	0.6	10	12
4	0.8	10	16
5	1.0	10	20
6	1.2	10	24

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.

Acceptance Criteria :

Correlation coefficient should not be less than 0.999.

d).Precision : Precision is the degree of repeatability of an analytical method under normal operating conditions.

Method Precision :In method precision a homogeneous sample of single batch should be analysed 6 times and calculate the % RSD. The precision of the instrument was checked by repeatedly injecting (n=6) solutions of 40ppm of Baricitinib).

Acceptance Criteria :The % RSD for the absorbance of 6 replicant injections results should not be more than 2%.

e).Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition.

i). Flow rate variation: The flow rate was varied at 0.9ml/min to 1.1ml/min standard solution of 20 ppm of Baricitinib was prepared and analysed using the varied flow rates along with method flow rates.

ii). **The variation of Organic Phase ratio**: Standard solution of 20ppm of Baricitinib was prepared and analysed using the varied in mobile phase ratio.

Results and Discussions:[9-13]

a).System precision: All the System suitability parameters were within the range and satisfaction as per ICH guidelines.

Table-2:system suitability parameters for Baricitinib

S.no	Parameter	Baricitinib
1	Retention time	3.714
2	Plate count	5634
3	Tailing factor	1.10
4	Resolution	-
5	%RSD	0.16

According to acceptance criteria, theoretical plates for the baricitinib peak in the standard solution is greater than 2000, as well as tailing factor and % of RSD is less than 2 and resolution is greater than 2.

b)Linearity:

Table-3: Results of linearity for Baricitinib

S.NO	Baricitinib	
	Conc.(µg/ml)	Peak area
1	20	459113
2	40	896209
3	60	1306347
4	80	1834505
5	100	2178517
6	120	2655617
Regression equation	y = 110213.88x +10334.54 0.99946	
Slope	110213.88	
Intercept	10334.54	
R^2	0.99946	

From the above table if the concentration increases or decreases the change occurred in areas linearly. Correlation coefficient is not less than 0.999.

c).Precision:

In method precision a homogeneous sample of single batch should be analyzed 6 times and calculate % RSD. This indicates whether a method is giving a constant result s for a single batch.

Table-4: Intermediate Precision for Baricitinib by RP-HPLC method

S.No.	Baricitinib peak area
1	1880847
2	1897995
3	1903999
4	1857151
5	1893482
6	1872710
Average	1884330.667
Standard deviation	16009.313
%RSD	0.849602104

Acceptance Criteria:

%RSD should be less than 2. The %RSD was found to be within the range.

d). Robustness:

Table-5: Robustness results for Baricitinib

Parameter	Baricitinib				
	Condition	**Retention time(min)**	**Peak area**	**Tailing**	**Plate count**
Flow rate Change (mL/min)	Less flow(0.9ml)	4.319	2270105	1.13	6856
	Actual(1ml)	3.726	1891923	1.10	5632
	More flow(1.1ml)	3.132	1562279	1.08	4361
Organic Phase change	Less Org (36:64)	4.331	1939870	1.11	5678
	Actual(40:60)	3.728	1895508	1.12	5639
	More Org (44:56)	3.118	1633129	1.06	5012

On the evaluation of the above results, it can be calculated that the variation in flow rate effected the method significantly. Hence, it indicates that the method is robust. Even by change in the flow rate ±10%.

Summary and conclusion :

Summary:

An attempt has been made to develop a validated stability indicating RP-HPLC method for the estimation of Baricitinib. Literature survey revealed that many analytical methods have been reported individually or in combination with other drugs.

Conclusion:

The developed HPLC method for the estimation of baricitinib is simple, rapid, accurate, precise, Robust and economical. The mobile phase and solvents are simple to prepare and economical, reliable, sensitive and less time consuming.

The sample recoveries were in good agreement with their label claims and we suggested noninterference of formulation excipients in the estimation and can be used in laboratories for the routine analysis of baricitinib. Since the system validation parameters of HPLC method used for estimation of selected drug in pure and have shown satisfactory, accurate and reproducible results (without any interference of excipients) as well, it is deduced that the simple and short proposed methods be most useful for analysis purpose.

The present work concluded that stability indicating assay method by RP-HPLC was simple, accurate, precise, and specific and has no interference with the placebo and degradation products. Hence these can be used for routine analysis of Baricitinib.

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