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METHOD DEVELOPMENT AND VALIDATION OF STABILITY INDICATING BARICITINIB BY RP-HPLC FOR BULK AND SOLID DOSAGE FORM

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ABSTRACT
The aim of study is to develop method and validation of Baricitinib by using RP-HPLC for bulk solid dosage form. Main objective is to develop and validate a new, simple, rapid, precise, and accurate An Eco-friendly RP-HPLC and UV-Method Development and Validation for an estimation of Baricitinib in Bulk and solid dosage form.To perform all the validation parameters according to ICH guidelines**.** Baricitinib belongs to class III of biopharmaceutical classification system (BCS), which means that it is highly soluble and poorly permeable drug, however, the same report also mentioned that baricitinib is practically insoluble. The analysis of the drug was carried out on Column: Phenomenex ODS-3,Column Dimension: (250 mm X 4.6 mm i.d.) 5µm Column Oven temperature: 35°C, Injection Volume: 20 µl, Wavelength: 251 nm, Mobile phase: Acetonitrile : 0.1% OPA in Water (60:40), Flow Rate: 1.0 ml/min, run time : 6 min having software open lab EZ chrome. 20 µg/mL as working concentration was used. Assay study was performed on Covinib 4mg tablet. Validation parameters like accuracy, precision, repeatabilitystudies, specificity, solubility, LOD, LOQ and stability testing was performed on Baricitinib. From the calibration curve it was concluded that the Baricitinib shows linear response in the range of 2.0-30.0 µg /ml. All the parameters were within range hence above method is validated.

KEYWORDS: Baricitinib, HPLC, Accuracy, stability, specificity, linearity, filtration etc.

INTRODUCTION

HPLC

- High performance liquid chromatography (HPLC) is an important qualitative and quantitative technique, generally used for the estimation of pharmaceutical and biological samples.
- It is the most versatile, safest, dependable and fastest chromatographic technique for the quality control of drug components. This article was prepared with an aim to review different aspects of HPLC, such as principle, types, instrumentation and application.
- High performance liquid chromatography (HPLC) is an important qualitative and quantitative technique, generally used for the estimation of pharmaceutical and biological samples. It is the most versatile, safest, dependable and fastest chromatographic technique for the quality control of drug components.

There are 2 types of HPLC

1.Reversed phase chromatography :- Reversed phase HPLC (RP-HPLC or RPC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase.

RPC operates on the principle of hydrophobic interactions, which result from repulsive forces between a polar eluent, the relatively non-polar analyte, and the non-polar stationary phase. The binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand in the aqueous eluent.

2. Normal phase chromatography:- Also known Normal phase HPLC (NP-HPLC), this method separates analytes based on polarity.
NP-HPLC uses a polar stationary phase and a non-polar mobile phase.
The polar analyte interacted with and is retained by the polar stationary phase.
Adsorption strengths increase with increased analyte polarity, and the interaction between the polar analyte and the polar stationary phase increases the Elution time.

Analytical method validation Method validation is a documented evidence which provides a high degree of assurance for a specific method that the process used to confirm the analytical process is suitable for its intended

use. The developed HPLC method for estimation ceftriaxone sodium was validated as per ICH Q2 (R1) guidelines.

Mechanism of action

- Baricitinib belongs to **class III** of biopharmaceutical classification system (BCS), which means that it is highly soluble and poorly permeable drug, however, the same report also mentioned that baricitinib is practically insoluble.
- As members of the tyrosine kinase family, Janus kinases (JAKs) are intracellular enzymes that modulate signals from cytokines and growth factor receptors involved in hematopoiesis, inflammation, and immune cell function.
- Upon binding of extracellular cytokines and growth factors, JAKs phosphorylate and activate Signal Transducers and Activators of Transcription.
- STATs modulate intracellular activity, including gene transcription of inflammatory mediators that promote an autoimmune response.
- Baricitinib is a selective and reversible inhibitor of JAK1 and JAK2 with less affinity for JAK3 and TYK2.
- It was also shown to decrease the proliferation of JAK1/JAK2 expression in mutated cells and induce cell apoptosis.

Drug Profile
Drug name :- Baricitinib
Drug category :-Antineoplastic, immunodilating.

Structure

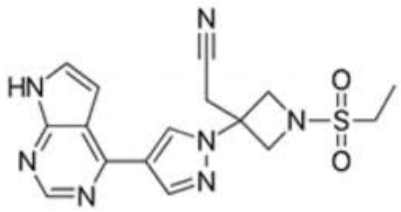


Fig. 1: Structure of Baricitinib.

IUPAC Name:- 2-[1-(ethanesulfonyl)-3-(4-{7H-pyrrolo[2,3-d]pyrimidin-4-yl}-1H-pyrazol-1-yl)azetidin-3-yl]acetonitrile

Molecular Formula :- $C_{16}H_{17}N_7O_2S$

Molecular weight :- 371.4

CAS NO :- 1187595-84-1

Melting Point :- 213–215 ºC

Solubility :- soluble in organic solvents such as dimethyl sulphoxide (74 mg/ml), dimethyl formamide (50 mg/ml) but very poorly soluble in water and ethanol (<1 mg/ml at 25 °C)

pKa :- 13.89

Physical State :- White crystals or powder

Storage condition :-Store the medicine in a closed container at room temperature, away from heat, moisture, and direct light. Keep from freezing.

MATERIALS AND METHODS
a.Selection of stationary phase: The column used in this method C_{18} Phenomenex ODS-3
b.Solubility Studies: Baricitinib is slightly soluble in water.

1. Selection of stationary phase and mobile phase.
2. Selection of chromatographic condition.
3. Study of system suitability parameter
4. Determination of Baricitinib by proposed method
5. Application of proposed method for estimation of marketed formulation.
6. Validation of Proposed method by using following parameters

- Accuracy
- Precision
- Linearity and range
- System suitability parameter
- Robustness
- Limit of detection
- Limit of quantitation
- Rugedness
- Specificity/selectivity
- Compilation of data

RESULTS AND DISCUSSION
1. PRELIMINARY CHARACTERIZATION AND IDENTIFICATION OF DRUG
Color, Odour and Appearance of Drug.

Sr. No	Name	Colour, odour and appearance of drug
1	Baricitinib	White, odourless and Crystalline powder.

2. Melting point

Sr. No.	Name	Melting point std. value (°C)	Melting point observed (°C)
1	Baricitinib	213-215 °C	214-216 °C

3. Solubility study of Baricitinib
Check solubility in water and methanol.

3. Selection of solvent
Methanol was selected as the solvent for dissolving Baricitinib.

5. Selection of analytical wavelength:- 251nm

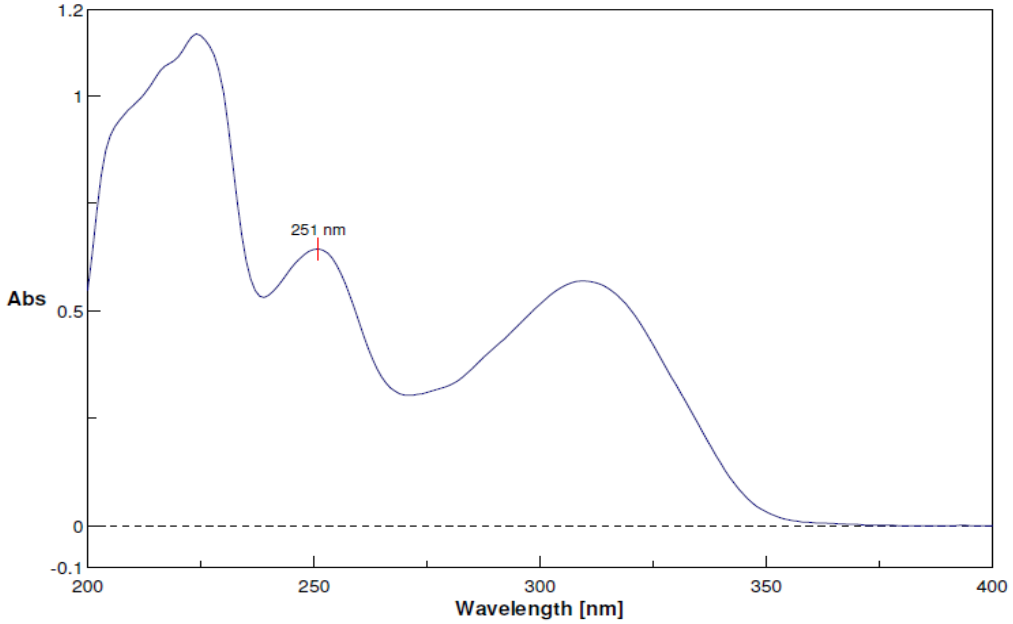


Fig. 2: UV spectrum of Baricitinib.

6. Method Development by RP – HPLC
Optimization of HPLC method

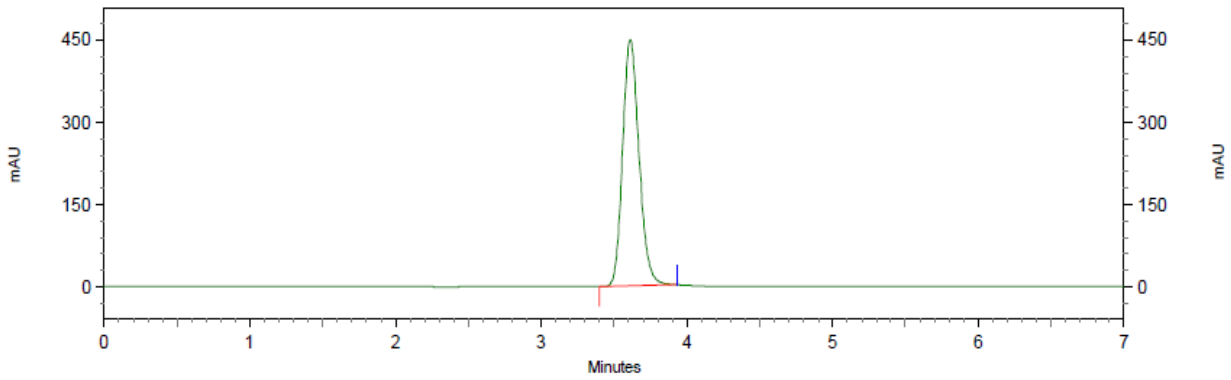


Fig.3: Typical chromatogram of Trial 5.

Observation: Baricitinib eluted with Good Chromatography. (Asymmetry: 1.16 & Theoretical plate: 8532).
Conclusion: Method Accepeted

1. Analysis of Marketed Test samples (Assay)
a) Covinib 4 mg Tablet
Weight of 20 tablets = 6.2080 gm
Average weight of tablet = 6.2080 / 20 = 0.3104 gm = 310.4 mg

Sample	Area	% Assay	Mean Assay
Sample 1	8360142	99.06	98.53
Sample 2	8269810	98.01	

Fig. 4: Assay results of Covinib 4 mg Tablet.

7. VALIDATION OF RP-HPLC METHOD
1. FILTRATION STUDY
Results of Filter study

Sample description	Area	% Absolute difference
Unfiltered	8460347	NA
0.45 µ PVDF filter	8439773	0.24
0.45 µ Nylon filter	8427546	0.39

3. Linearity and Range

Level	Conc (µg/mL)	Area	Mean	% RSD
10%	2.00	847269	**845421**	**0.256**
		845960		
		843034		
50%	10.00	4349612	**4336660**	**0.308**
		4322917		
		4337452		
100%	20.00	8447316	**8427969**	**0.240**
		8406975		
		8429617		
125%	25.00	10613049	**10583083**	**0.319**
		10589716		
		10546483		
150%	30.00	12686173	**12642377**	**0.316**
		12633047		
		12607910		

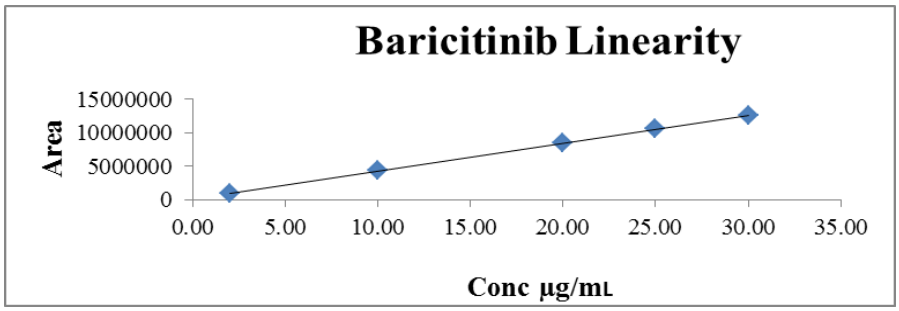


Fig. 5: Calibration curve of Baricitinib.

Sr no.	Parameter	Result value	Acceptance criteria
1	Beer's linearity range	2.0-30 µg/mL	NA
2	Correlation coefficient (R^2)	0.99994	NLT 0.98
3	Intercept	54261.17	To be report
4	Slope	420278.2085	To be report
5	% RSD for area at each level	NA	NMT 2.0

The respective linear equation for Baricitinib was:
Y = M X + C
Y = 420278.2085 x + 54261.17

CONCLUSION
From the calibration curve it was concluded that the Baricitinib shows linear response in the range of 2.0-30.0 µg /ml. The Regression value was found well within the limit.

4. Limit of Detection (LOD) and Limit of Quantitation (LOQ)
σ = 51674.69 (Residual standard deviation of a regression line)
s = 420278.2085 (Slope)
Detection limit (LOD):
LOD = 3.3 σ / S
LOD = 3.3 x 51674.69 / 420278.2085
LOD = 0.406 µg/mL

5. Quantitation limit (LOQ)
LOQ = 10 σ / S
LOQ = 10 x 51674.69 / 420278.2085
LOQ = 1.230 µg/mL

6. ACCURACY (RECOVERY): The accuracy of an analytical method is the closeness of test results obtained by that method to the true value.

Level (%)	Area	Recovered conc (µg/mL)	Added conc (µg/mL)	% Recovery	Mean Recovery	% RSD
50	4326898	10.26	10.30	99.61	98.95	0.6477
	4212564	9.99	10.10	98.91		
	4229913	10.03	10.20	98.33		
100	8410147	19.94	20.20	98.71	99.32	0.5999

	8469713	20.08	20.10	99.90		
	8465893	20.07	20.20	99.36		
150	12645131	29.97	30.10	99.57	99.79	0.7852
	12589304	29.84	30.10	99.14		

Fig. 6: Result and statistical data of Accuracy of Baricitinib.

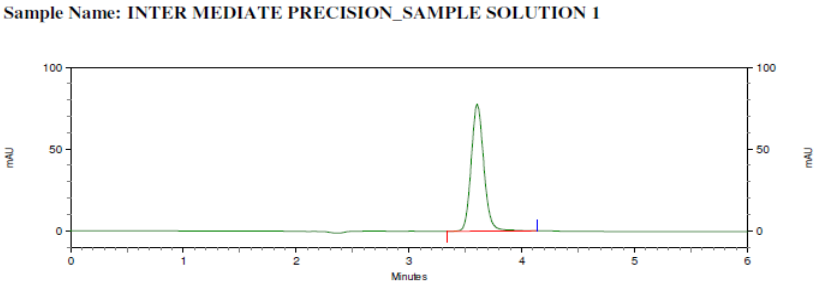
	Sample	**Test Sample (mg)**	**Area**	**% Assay**
Repeatability	Sample 1	1552.2	8334675	98.77
	Sample 2	1552.4	8407130	99.62
	Sample 3	1551.9	8330671	98.74
	Sample 4	1552.1	8219271	97.41
	Sample 5	1551.8	8361079	99.11
	Sample 6	1552.1	8467900	100.36
	Mean			99.00
	STD DEV			0.9894
	% RSD			0.999
Intermediate precision (Inter-Day)	Sample 1	1551.9	8269142	98.01
	Sample 2	1552.1	8196140	97.14
	Sample 3	1552.3	8321291	98.61
	Sample 4	1552.2	8404964	99.60
	Sample 5	1552.4	8379643	99.29
	Sample 6	1551.8	8261304	97.93
	Mean			98.43
	STD DEV			0.9199
	% RSD			0.935
Repeatability Plus Inter-day	Mean			98.716
	STD DEV			0.9585
	% RSD			0.971

Overall Recovery: 99.35 %
% RSD for Overall Recovery: 0.696

Acceptance criteria
% Recovery for each level and overall recovery: 98.0 to 102.0%
% RSD for each level and overall recovery: NMT 2.0
Data interpretation: Recovery of analytical procedure was found well within acceptance criteria at all 3 levels. % Recovery not get hampered by changed in analyte concentration.

7. PRECISION:- Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogenous sample.

Result of Intra- day and Inter- Day Precision for Baricitinib test sample assay



VWD: Signal A, 251 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Baricitinib	3.61	8269142	1.18	8809
Totals		8269142		

Fig. 7: Typical chromatogram of Inter-day precision (Sample 1).

% Assay: % Assay value for each sample (Individual sample) and mean assay value for precision (6 sample), mean assay value intermediate precision (6 sample), and mean assay value for precision plus intermediate precision sample (12 sample): 90-110%

% RSD: % RSD for precision study samples(6 sample), Intermediate precision study samples (6 sample) and precision plus intermediate precision sample (12 sample): NMT 2.0

Data interpretation: % Assay and % RSD was found well within acceptance limit and hence method is precise (Reproducible).

8. ROBUSTNESS

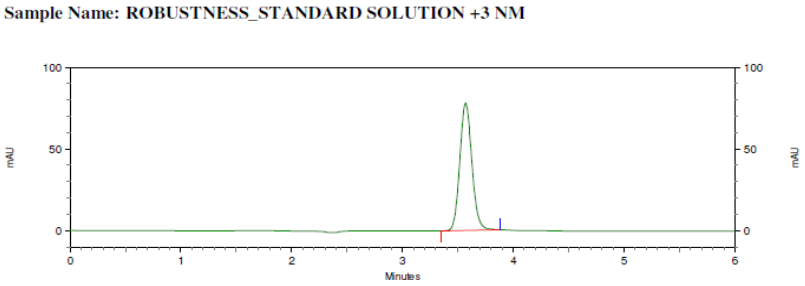
The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Following changes made under Robustness:
Change in Wavelength
Change in flow rate
Change in column oven temperature

Change in Parameter	R.T.	Standard area	Asymmetry	Theoretical plates
Wavelength by +3 NM (254 NM)	3.58	8269716	1.16	8612
Wavelength by -3 NM (248 NM)	3.57	7539612	1.16	8816
Flow rate by +10% (1.1mL/min)	3.24	8069034	1.14	8456
Flow rate by -10% (0.9mL/min)	3.96	8602534	1.15	9025
Column oven temp by +2ºC (37 ºC)	3.60	8329716	1.19	8876
Column oven temp by -2ºC (33 ºC)	3.61	8409626	1.16	8651

Fig.8 Result of Robustness study.

1. Change in Wavelength by +3 NM:
2. Change in Wavelength by -3 NM
3. Change in Flow rate by + 10% (1.1 mL/min)
4. Change in Flow rate by - 10% (0.9 mL/min)
5. Change in Column Oven temperature by +2°C
6. Change in Column Oven temperature by -2°C



VWD: Signal A, 254 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Baricitinib	3.58	8269716	1.16	8612
Totals		8269716		

Fig. Typical chromatogram of Standard +3 NM.

Acceptance criteria: Chromatography (System suitability) acceptance criteria should not get failed.
Data interpretation: From the above results, it was concluded that the system suitability test result was found well.

CONCLUSION

Methods (RP-HPLC single - component mode of analysis) have been developed and validated for determination of Baricitinib

RP-HPLC methods are found to be accurate, precise, rugged and robust.

All these methods are adequately sensitive.

The only limitation of RP HPLC method is its cost.

UV-spectrophotometric methods are simple, accurate and economical and least calculations are involved for estimation of concentrations of both these drugs.

All the developed methods can be used for routine analysis of these drugs in their combined pharmaceutical formulation.

ACKNOWLEDGEMENT
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