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Analytical Method Development and Validation of RP-HPLC for The Quantitative Determination of Baricitinib in Pure Substances and Marketed Formulation

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Abstract
An accurate, precise and rapid RP-HPLC method was developed and subsequently validated for the determination of Baricitinib in bulk form and marketed pharmaceutical dosage form. Better separation of the drug was achieved on Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d. with the mobile phase consisted of mixture of Methanol and Phosphate buffer (0.02M, pH-3.6) in ratio of 45:55v/v at flow rate of 1.0ml/min, with detection at 255nm using UV detector. The retention time was found to be 3.254min. The method was found to be linear in the range of 12-28µg/ml with a correlation coefficient (r2) of 0.9995. The LOD and LOQ of the method were calculated to be 5.004 and 15.164µg/ml respectively. The Precision was estimated by employing repeatability; intra-day and inter-day studies and the results were calculated as %RSD values and were found to be within the limits. Recovery of Baricitinib was found to be in the range of 98-102-% which confirms the accuracy of the method. The proposed HPLC method is validated using standard ICH guidelines.

Keywords
Baricitinib, RP-HPLC, Method Development, Accuracy, Precision, ICH Guidelines.

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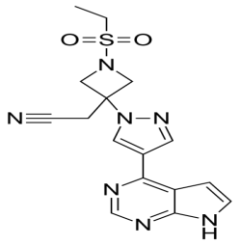
INTRODUCTION:

Baricitinib

The Chemical Formula is $C_{16}H_{17}N_7O_2S$. 371.42g/mol: **Molecular Weight**

Structure



2- [1-ethyl sulfonyl-3-[4-(7H-pyrrolo [2, 3-d]] **IUPAC Nomenclature** pyrimidin-4-yl) pyrazol-1-yl] azetidin-3-yl] acetonitrile

Mechanism of Activation: Janus kinases (JAKs) are intracellular enzymes that regulate cytokine and growth factor receptor signals during hematopoiesis, inflammation, and immune cell activity. They belong to the tyrosine kinase family. When cyto-kines & progress influences drag to JAKs, they phosphor-rylate & trigger Pointer Trans-ducers & Activ-ators of Transcript (STATs). STATs control intra/cellular activity, together with gene transcription of inflammatory mediators such as IL-2, IL-6, IL-12, IL-15, IL-23, IFN-, GM-CSF, and interferons, all of which cause an autoimmune response. The JAK-STAT pathway, which is associated to inflammatory mediator overproduction, takes been allied to the pathogenesis of rheumatoid arthritis.

MATERIALS AND METHODS:

Chemicals and Reagents: Methanol, Ethanol, Acetone, Dimethyl sulphoxide, Phosphate buffer.

Instruments:

Waters HPLC with **Empower** software, Electronic Balance **(CITIZEN BALANCE BL-220H),** Ultra Sonicator **(ANALYTICAL),** and P^H Analyzer **(ELICO),** Distillation unit **(BOROSIL),** Vaccum filtration unit **(BOROSIL).**

Reagents and Solutions

Selection of Wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent. (After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Baricitinib, so that the same wave number can be utilized in HPLC UV detector for estimating the Baricitinib.

Preparation of Standard and Sample Solutions

25 mg of Baricitinib standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.3 ml of the above solution into a 10 ml volumetric flask and make up to volume with mobile phase.

Preparation of 0.02M Potassium di hydrogen orthophosphate Solution

About 2.72172grams of Potassium di hydrogen orthophosphate was weighed and transferred into a 1000ml beaker, dissolved, and diluted to 1000ml with HPLC Grade water. The pH was adjusted to 2.80 with diluted ortho phosphoric acid Solution.

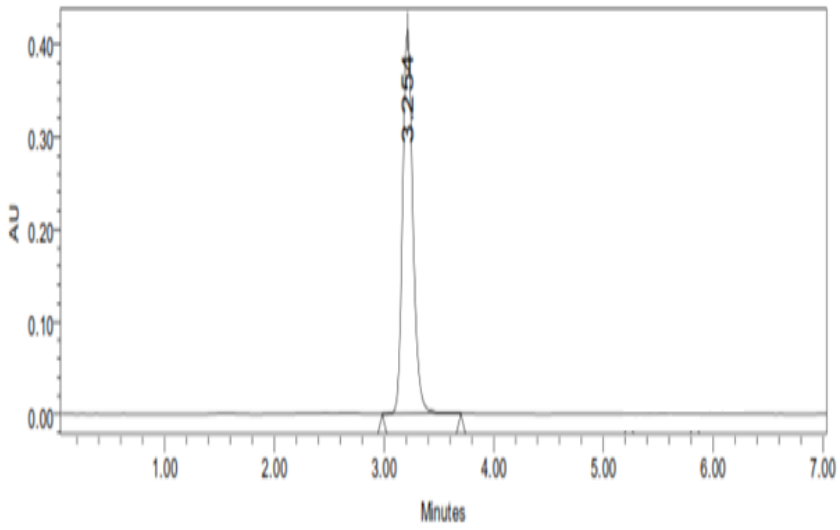
Preparation of Mobile Phase

550 mL (55%) of above Phosphate buffer solution and 450 mL of Methanol (45%) were mixed well and degassed in ultrasonic water bath for 15 minutes. The resulted solution was filtered through 0.45 µm filter under vacuum filtration.

METHOD VALIDATION

Method Optimization:

Mobile phase	Methanol : Phosphate buffer (0.02M, pH-3.6) = 45:55
Column	Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.
Column Temperature	Ambient
Detection Wavelength	255 nm
Flow rate	1.0 ml/ min.
Run time	07 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	20µl
Type of Elution	Isocratic



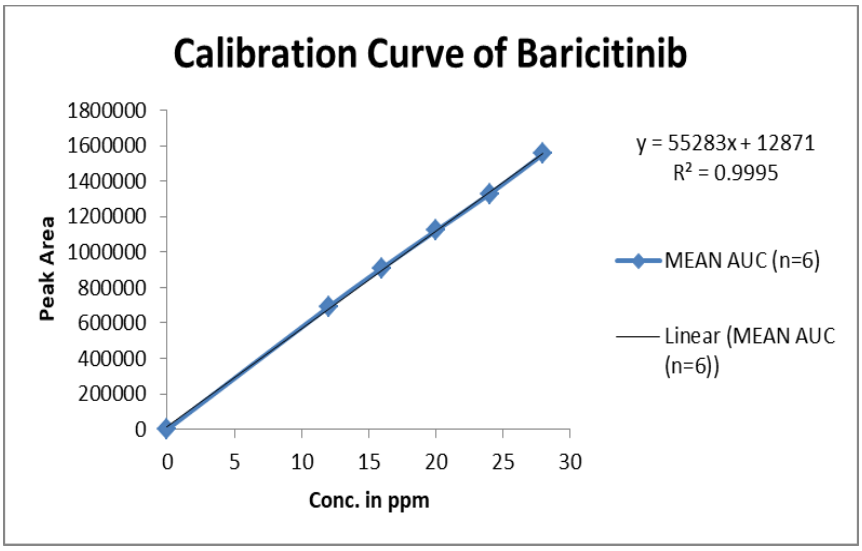
Optimized Chromatographic Condition

Results of Optimized Chromatogram

S. No.	Drug Name	RT	Peak Area	Theoretical Plates	Tailing Factor
1	Baricitinib	3.254	283261	7258	1.25

RESULTS AND DISCUSSION:

Standard Curve for Baricitinib



Linearity Readings for Baricitinib

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
12	690316
16	910621
20	1121057
24	1328903
28	1554666

Table-1: Results of accuracy:
Accuracy outcomes of Baricitinib

Sample ID	Concentration (µg/ml)			%Recovery of Pure drug	Statistical Analysis
	Conc. Found	Conc. Recovered	Peak Area		
S_1 : 80 %	8	8.064107	458679	99.867	Mean= 100.4113%
S_2 : 80 %	8	7.843532	446485	100.637	S.D. = 0.473694346
S_3 : 80 %	8	8.19449	465887	100.73	% R.S.D.= 0.471753
S_4 : 100 %	10	9.892661	559767	99.41	Mean= 100.6646667%
S_5 : 100 %	10	9.978655	564521	100.868	S.D. = 1.166369295
S_6 : 100 %	10	10.19623	576549	101.716	R.S.D.= 1.158667
S_7 : 120 %	12	11.85907	668476	99.878	Mean= 100.4637%
S_8 : 120 %	12	12.16785	685546	100.69	S.D. = 0.51154309
S_9 : 120 %	12	12.18644	686574	100.823	% R.S.D. = 0.509181

2. Precision:
Repeatability Results of Baricitinib

HPLC Injection Replicates	AUC for Baricitinib
Replicate – 1	285479
Replicate – 2	284571
Replicate – 3	286954
Replicate – 4	283261
Replicate – 5	285964
Replicate – 6	284259
Average	**285081.3**
Standard Deviation	**1318.666**
% RSD	**0.462558**

ii) Intermediate Precision / Ruggedness

Ruggedness Results for Baricitinib

Conc. of Baricitinib (API) (µg/ml)	Observed Conc. of Baricitinib (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=3)	% RSD	Mean (n=3)	% RSD
8	8.21	0.76	8.23	0.46
10	10.37	0.33	10.36	0.57
12	12.56	0.23	12.56	0.75

Robustness:

Consequence of Technique Toughness

Change in Parameter	% RSD
Flow (0.8 ml/min)	0.554
Flow (1.2 ml/min)	0.867
More Organic	0.886
Less Organic	0.817
Wavelength of Detection (257 nm)	0.813
Wavelength of detection (253 nm)	0.794

LOD: For Baricitinib, the LOD was determined to be 5.004g/ml.

LOQ: The LOQ for Baricitinib was discovered to be 15.164g/ml.

Assay:

Retrieval Statistics for Assessment Baricitinib in Olumiant Tablets

Brand Name of Baricitinib	Labelled amount of Drug (mg)	Mean (± SD) amount (mg) found by the proposed method (n=6)	Assay % (± SD)
Olumiant Tablet (Lilly)	4mg	3.896 (± 0.867)	99.698 (± 0.476)

FORCED DEGRADATION STUDIES
Consequences of Enforced Deprivation

Stress Condition	Time (hours)	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1N HCl)	24Hrs.	91.326	8.674	100.00
Basic Hydrolysis (0.IN NaOH)	24Hrs.	83.215	16.785	100.00
Thermal Degradation (60 ^{0}C)	24Hrs.	90.311	9.689	100.00
UV (254nm)	24Hrs.	81.322	18.678	100.00
3% Hydrogen Peroxide	24Hrs.	73.514	26.486	100.00

CONCLUSION:

This study created a simple, quick, efficient, and reliable RP-HPLC method for assessing Baricitinib in bulk medication and pharmaceutical dosage forms. This procedure was straightforward since diluted samples were employed immediately without any chemical derivatization or purification. The travelling segment was a 45:55 percent v/v mixture of methanol and phosphate buffer. This procedure's solvent system was proved to be cost-effective. The approach was validated, and the percent RSD results were accurate to within a factor of two. The RP-HPLC technique yielded good results, as shown in the tables. Spectrophotometric technologies are less sensitive, accurate, and precise than RP-HPLC technology. This approach might be used on a regular basis to assess the concentration of Baricitinib in bulk medicine and pharmaceutical dosage forms.

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