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Development and Validation of a Stability indicating Related Substances of Baricitinib by RP-HPLC and its Degradation

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Abstract*: Reverse phase high performance liquid chromatography method, for estimation of related substances or chromatographic impurities of Barcitinib was developed and validated. Baricitinib was developed by separating its degradation products on a X-Terra RP18 (150x4.6mm, 5.0 µm) column using 0.1% Tri ethyl amine in water adjusted pH-2.5 with OPA and Acetonitrile in simple gradient at a flow rate 1.0 ml/min. The column effluents were monitored by a photodiode array detector set at 224nm. The method was validated in terms of specificity, linearity, accuracy, precision, detection limit, quantification limit and robustness. Forced degradation of Baricitinib was carried out under acidic, basic, peroxide, reduction, thermal, photo and hydrolysis conditions. The proposed method is validated as per ICH Q2 (R1) guidelines. The proposed method is simple as selected chromatographic conditions are not so difficult to apply in routine analysis for testing the chromatographic impurity of baricitinib.

Keywords: Baricitinib, RP-HPLC, Related substances, Chromatographic impurity.

I. INTRODUCTION

Baricitinib is a drug for the treatment of rheumatoid arthritis [1] being developed by Incyte [2] and Eli Lily [3]. Baricitinib is an orally bio available inhibitor of Janus kinases [4] 1 and 2 (JAK1/2), with potential anti-inflammatory, immunomodulating and antineoplastic [5]activities. Upon administration, baricitinib binds to JAK1/2 activation and leads to the inhibition of the JAK-signal transducers and activators of transcription (STAT) signaling pathway. This decreases the production of inflammatory cytokines [6] and may prevent an inflammatory response. In addition baricitinib may induce apoptosis [7] and haematopoiesis [8] they are also unregulated and/or mutated in various tumor [9]cell types.

Baricitinib is an orally bio available small molecule inhibitor of Janus kinases [10] that is used to treat moderate-to-severe rheumatoid [11] arthritis. Baricitinib is associated with transient and usually mild elevations in serum aminotransferase [12] levels during therapy but has yet to be linked to cases of clinically apparent acute liver injury.

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II. MATERIALS AND REQUIREMENTS

Instrument

HPLC, make: Waters alliance e-2695 chromatographic system consisting of quaternary pump, PDA detector-2996 and chromatographic software Empower-2.0 was used.

Reagents

CH_3CN(HPLC grade), H_3PO_4(HPLC grade), H_2O (HPLC grade).

Mobile Phase Preparation

Mobile Phase-A: 1ml Orthophospharic acid is transferred into 1lt water. Filter through membrane (0.45µ) and degas.
Mobile Phase-B: CH_3CN
Preparation of diluent: 50:50 v/v. of mobile phase-A and mobile phase-B were mixed.

Optimization of mobile phase

Different trails have done, different buffers and different mobile phases were used to develop the method. In all trails peaks are not separated properly. Finally for the proposed method all the peaks are separated and the entire suitability conditions are within the limit.

Table-I: Gradient Program

Time (min)	Mobile Phase-A	Mobile Phase-B
0.00	80	20
5	50	50
7	50	50
8	80	20
12	80	20

Chromatographic conditions

The chromatographic system was carried out in symmetry C18, (150x4.6mm, 3.5µm) column. Flow rate was maintained at 1.0ml/min injection volume is 10µl and sample and column temperatures are ambient. Wavelength detection is maintained at 265 nm.

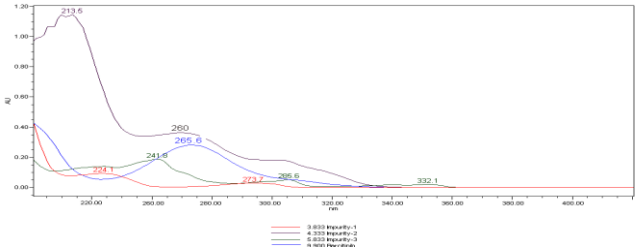

Fig. 1. PDA Spectra for Baricitinib and its impurities Standard Solution

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Accurately weigh Baricitinib (100.3mg). The working standard is transferred into a volumetric flask(100ml) add 70ml of 50:50 v/v. of mobile phase-a and mobile phase-b sonicated for 10min to dissolve the contents make up to the mark with 50:50 v/v. of mobile phase-a, and mobile phase-b. Further 5ml of above solution to 50ml with diluent.

Sample Solution

Transfer 500.2mg of sample into a 100ml volumetric flask diluted to volume with diluent. Filter through 0.45µ nylon syringe filter.

Impurity standard stock solution

Weigh accurately impurity-1(50mg), impurity-2(250mg) and impurity-3 (100mg) and taken into a 100ml volumetric flask. Add 70ml of 50:50 v/v. of mobile phase-a, and mobile phase-b, sonicated to dissolve and make up.

Spiked Sample Solution

Transfer 10ml of sample into a volumetric flask (100ml) add add 1ml of impurity standard stock solution and 70ml of makeup to the mark with 50:50 v/v. of mobile phase-a, and mobile phase-b. Filter through 0.45µ syringe filter.

III. RESULTS AND DISCUSSION

Validation of proposed method

The proposed method was validated for parameters like, specificity, linearity, LOD, LOQ, precision, accuracy, robustness and ruggedness as per ICH guidelines [13-14].

System Suitability

The HPLC system was stabilized for 60min to get a stable baseline. Six replicate injections of standard solution were injected. The results are summarized below Table II.

Table- II: System Suitability Data

System Suitability parameter	Acceptance criteria	Drug Name
		Baricitinib
% RSD	NMT 2.0	0.67
USP Tailing	NMT 2.0	1.03
USP Plate Count	NLT 3000	50637

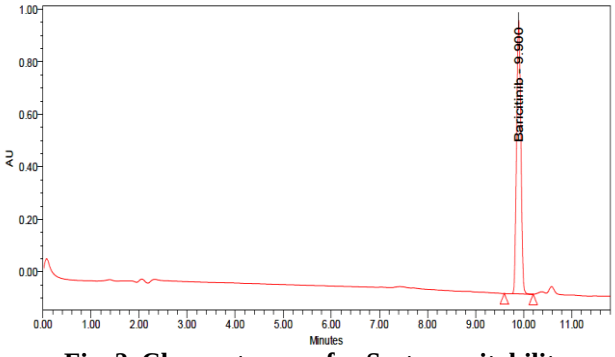

Fig. 2. Chromatogram for System suitability

Specificity

There is no interaction of peaks in blank and standard, sample, placebo chromatograms in the total runtime of chromatogram. Hence its proves that method is specific.

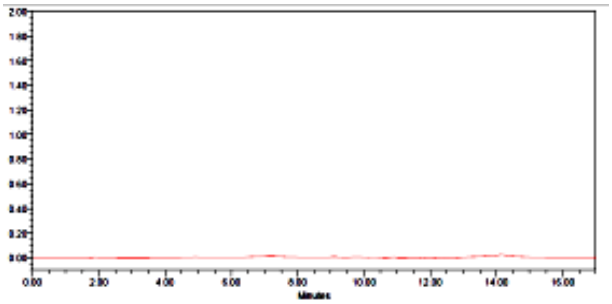

Fig 3. Chromatogram for Blan

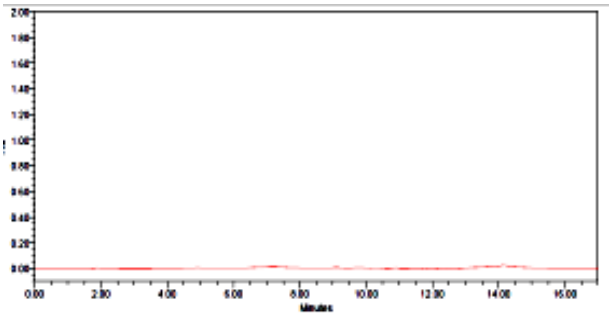

Fig 4: Chromatogram for Placebo

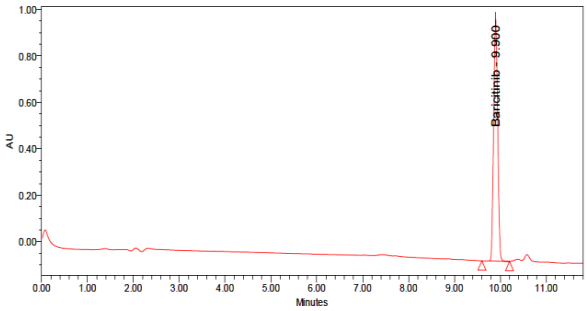

Fig 5: Chromatogram for Standard

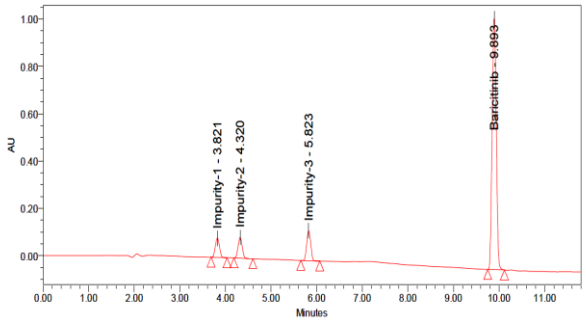

Fig6: Chromatogram for Sample

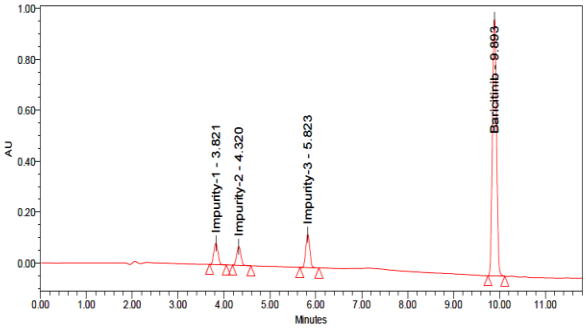

Fig 7: Chromatogram for spiked sample solution

Linearity

The linearity was observed in the concentration range of 10-150µg/ml for Baricitinib. The regression equation is Y=57232X+161136and correlation coefficient was found to be 0.99949. Impurity-1 concentration range from 0.5µg/ml to 7.5µg/ml, regression equation is Y=5170X+3724.8 and correlation coefficient were found to be 0.99918. Impurity-2 concentration range from 0.5µg/ml to 7.5µg/ml regression equation is Y=4502X+3574.5 and correlation coefficient were found to be 0.99933. Impurity-3 concentration range from 1-15µg/ml regression equation is Y=7577X+9140.4 and correlation coefficient was found to be 0.9995

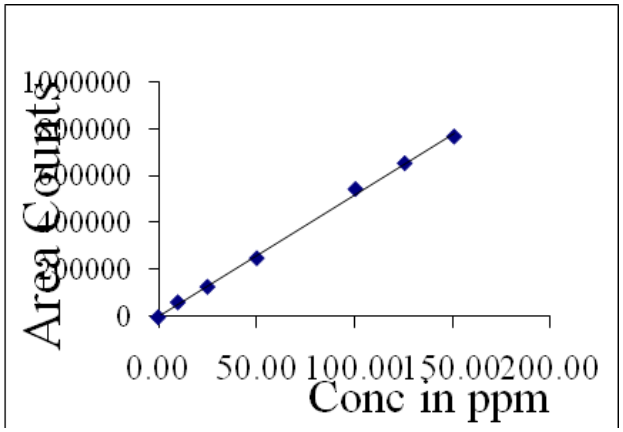

Fig 8: Linearity plot for Baricitinib

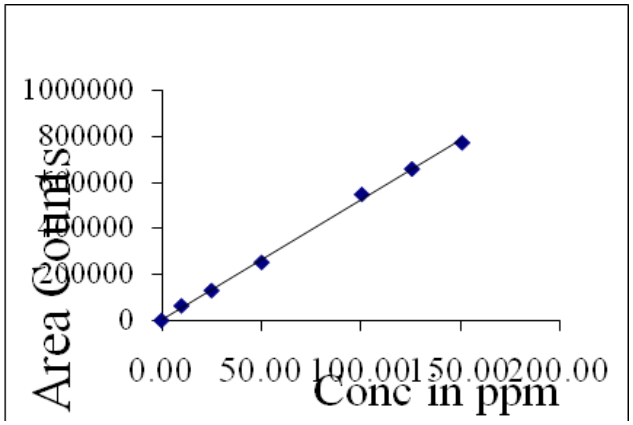

Fig 9: Linearity plot for Impurity-1

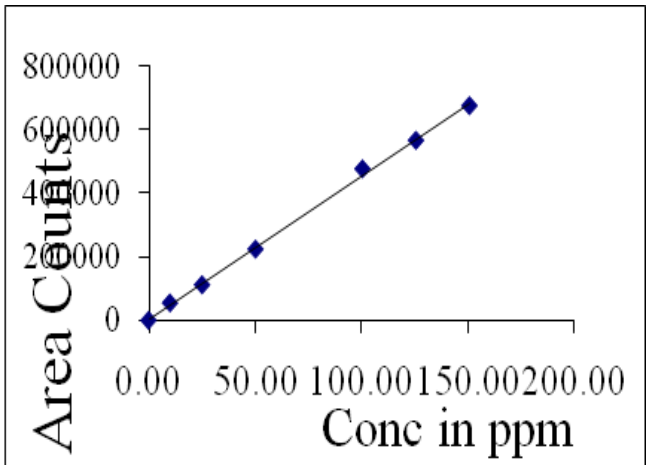

Fig 10: Linearity plot for Impurity-2

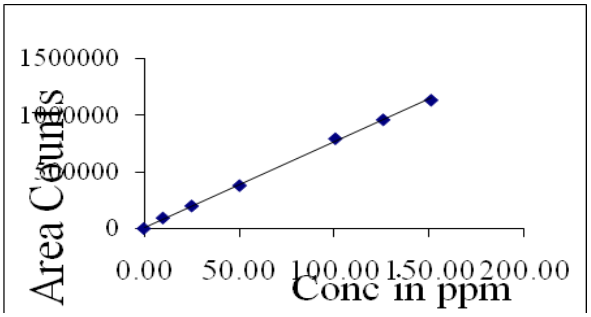

Fig 11: Linearity plot for Impurity-3

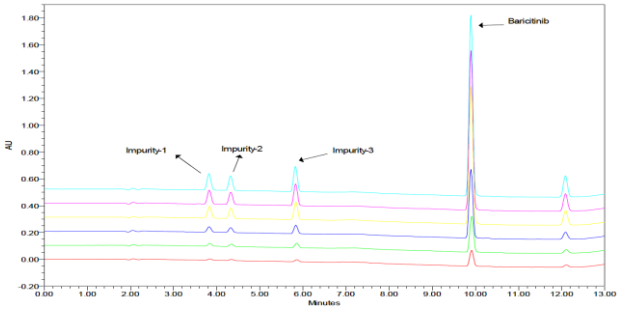

Fig 12: Overlay chromatogram for Linearity

Accuracy

The accuracy of the related substances test procedure was determined by spiking of Baricitinib and impurities stock solution to test the sample. So that the concentration of the impurity would be 0.5% of the test concentration as per the test method. Injecting samples in triplicate at 50%, 100% and 150% of the target concentration. The recovery results should be NLT 95.0% and NMT 105.0%.

Table -III: Accuracy results for Baricitinib

S.No.	% Level	% Recovery	Avg. %Recovery
1		100.4	
2		100.1	
3	50	100	100.2
4		100.3	
5		100.2	
6	100	99.9	100.1
7		100.5	
8		100.7	
9	150	100.1	100.4

Table IV: Accuracy results for Impurity-1

S.No.	% Level	% Recovery	Avg. %Recovery
1		100.2	
2		100.2	
3	50	100.6	100.7
4		99.9	
5		100.1	
6	100	100.3	100.1
7		100.1	
8		100	
9	150	100.8	100.6

Table V Accuracy results for Impurity-2

S.No.	% Level	% Recovery	Avg. %Recovery
1	50	100.3	100.1
2		99.9	
3		100.0	
4	100	100.6	100.5
5		100.2	
6		100.6	
7	150	100.7	100.5
8		100.7	
9		100.2	

Table VI : Accuracy results for Impurity-3

S.No.	% Level	% Recovery	Avg. %Recovery
1	50	100.3	100.3
2		100.5	
3		100.1	
4	100	99.9	100.1
5		100.2	
6		100.1	
7	150	99.9	100.1
8		100.1	
9		100.4	

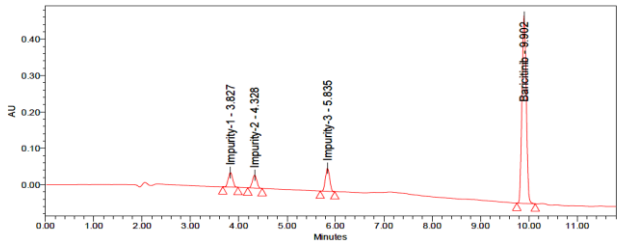

Fig 13: Chromatogram for Accuracy 50%

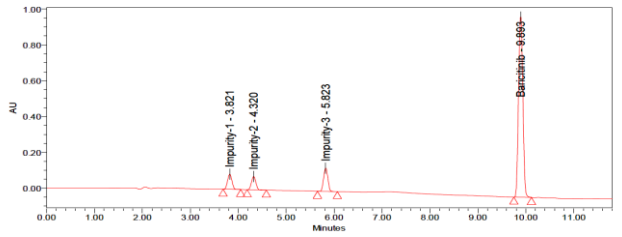

Fig 14: Chromatogram for Accuracy 100%

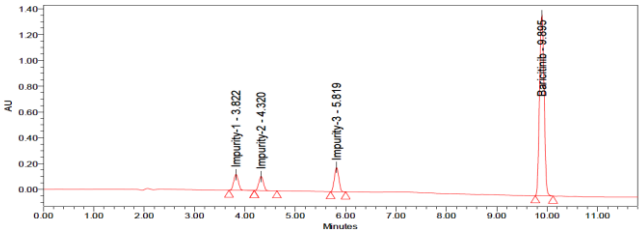

Fig 15: Chromatogram for Accuracy 150%

Precision

Precision of the test method was determined by repeatability assessed using a minimum of 6 determinations and calculated % relative standard deviation of impurities. The results were given in Table 7. Related substances results meet the specification limits.

Table VII: Precision results for Baricitinib

Sample No.	% of Related Substances	
	Total Impurities	% Purity (100-Total Imp.)
1	0.13	99.87
2	0.16	99.84
3	0.10	99.90
4	0.14	99.86
5	0.17	99.83
6	0.14	99.86
Average	0.14	99.88
%RSD	0.18	0.15

Intermediate Precision

Six replicates of a sample solution were analysed on a different day, different analyst and different instrument. Peak areas were calculated which were used to calculate mean, % RSD values. The results are given below table VIII

Table VIII: Precision results for Baricitinib

Sample No.	% of Related Substances	
	Total Impurities	% Purity (100-Total Imp.)
1	0.18	99.82
2	0.14	99.86
3	0.09	99.91
4	0.12	99.88
5	0.15	99.85
6	0.10	99.90
Average	0.15	99.85
%RSD	0.13	0.69

LOD and LOQ

LOD and LOQ were separately determined by calibration curve method[15]. LOD and LOQ of the compound were determined by injecting progressively lower concentrations of standard solutions using developed RP-HPLC method. The LOD concentrations for Baricitinib and their impurities-1, 2, 3 are 0.0515,0.0505,0.52,0.051µg/ml their s/n values are 3,3,4,7. The LOQ concentration for Baricitinib and their impurities-1, 2, 3 are 0.103,0.101,0.104,0.102 µg/ml their s/n values are 22,22,24,28.

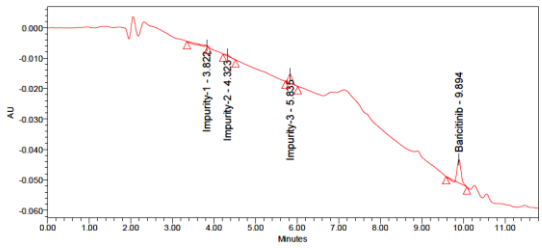

Fig 16: Chromatogram for LOD

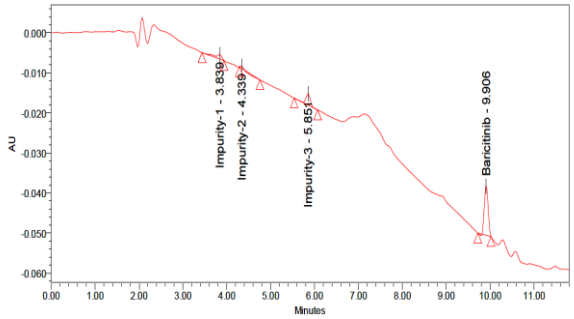

Fig 17: Chromatogram for LOQ

Robustness

The robustness of the method was evaluated by analyzing the system suitability standards and evaluating system suitability parameter data after varying the HPLC pump flow rate (±0.2 ml) and organic solvent content (±10%). The alterations caused a significant change in peak area R.S.D (%), USP tailing factor and retention times

Table 9: Robustness data

Parameter name	% RSD for Purity
	Baricitinib
Flow (0.8 ml/min)	0.155
Flow (1.2ml/min)	0.201
Organic solvent (+20%)	0.201
Organic solvent (-20%)	0.147

Stability

The stability of Baricitinib in solution was determined by sample solution stability initial to 24hr at different time intervals at room temperature and 2-8°C. There is no significant deviation of purity. Difference between initial to 24hr ±9.0%.

Table 10: Results for Solution Stability

Stability	Purity of Baricitinib	% of Deviation
Initial	99.99	0.00
12Hr RT	99.96	0.03
12Hr 2-8ºC	99.95	0.04
24Hr RT	99.98	0.01
24Hr 2-8°C	99.97	0.02

Forced Degradation

Acid Degradation:

10ml of sample transferred into a 100ml volumetric flask add 10ml of 0.1N HCl heat for 15min at 60ºC after that add 10ml of 0.1N NaOH then makeup to mark with diluent. Then the solution is filter through 0.45µ nylon syringe filter.

Alkali Degradation:

10ml of sample transferred into a 100ml volumetric flask add 10ml of 0.1N NaOH heat for 15min at 60ºC after that add 10ml of 0.1N HCl then makeup to the mark with diluent. Then the solution is filter through 0.45µ nylon syringe filter.

Peroxide Degradation:

10ml of sample transferred into a 100ml volumetric flask add 5ml of 10%H_2O_2 heat for 30min at 60ºC then cool to makeup with diluent. Filter the solution with 0.45µ nylon syringe filter.

Reduction Degradation:

10ml of sample transferred into a 100ml volumetric flask add 10ml of 10% sodium bicarbonate solution heat for 15min at 60ºC then cool to makeup with diluent. Filter the solution with 0.45µ nylon syringe filter.

Thermal Degradation:

The sample drug solution was placed in oven at 105ºC for 6Hr. The resultant solution was injected into HPLC system.

Photolytic Degradation:

The sample solution was exposed into sunlight for 6hr. The sample was injected into HPLC system

Table 11: Results for Forced Degradation

Degradation Condition	% of Purity
	Baricitinib
Acid Degradation	72.31
Alkali Degradation	72
Peroxide Degradation	72.13
Reduction Degradation	72.13
Thermal Degradation	72.18
Photolytic Degradation	72.07

IV. CONCLUSION

The developed method gave good resolution between Baricitinib and its 3-impurities with short runtime (12min), high efficiency and complies with modified SST specifications of USP. The use of C18 column in the present work has shown better elution of analytes with good resolution, improved plate count, tailing. So the C18 column can be used to achieve high specificity in shorter time of analysis of Baricitinib as per ICH Q3A (R2)[16] guidelines. The proposed method was found to be simple, precise, accurate, linear, robust and rapid for simultaneous determination and quantification of Baricitinib. The sample recovery was in good agreement with their respective label claims suggested non-interference in the estimation. Hence, the method can be easily and conveniently adopted for routine analysis of Baricitinib dosage form.

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