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Analytical Method Development and Validation for Estimation of Baricitinib in Bulk and Formulation

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KEYWORDS

High Performance Liquid Chromatography (HPLC), Anti-rheumatic, Baricitinib, Acetonitrile, trifluoroacetic acid, ICH guidelines, Validation.

ABSTRACT:

Baricitinib is an anti-rheumatic agent that is given orally. A simple, accurate, economical, and stable High-Performance Liquid Chromatography (HPLC) method was achieved for the analytical determination of Baricitinib in the tablet dosage form. HPLC systems of Thermo ultimate 3000 was specifically used for the development of the novel method for the drug determination. The chromatographic separation of the selected compound was carried out using Eurosphere C18 column (250mm x 4.6mm i.d.5µ). The defined mobile phase selected was Phosphate buffer (pH 6.5): Acetonitrile associated in the ratio of 70:30 v/v. The flow rate was set at 1.5 ml/minutes with column temperature maintained at 40º C and the injection volume of 20µl. The detection was estimated at 306 nm with the help of UV-VIS detector. The retention time was further observed at 4.9 minutes with the overall run time of approximately 10 minutes. The developed method was found to be linear over the range of 10 to 75 µg/ml with a linear regression coefficient (r^2) of 0.9996. The projected technique was found to be within the acceptable limits and with excellent results. The developed HPLC method for best suited with the validation parameters such as robustness, specificity, rapidity, reproducibility, and superior suitable parameter. The HPLC method was validated as per ICH guidelines. Hence the method can be used for the routine analysis of Baricitinib in bulk and tablet.

1. Introduction

Rheumatoid arthritis is an autoimmune inflammatory disease which commonly means that the immune system attacks the healthy cells of the body by mistakes that results in the painful swelling or inflammation in the affected parts of the body. It mainly attacks the joints, also several joints at once.

Baricitinib is a small molecule that is developed by Incyte and Eli Lily and approved by EU and Japan for the treatment of rheumatoid arthritis in adults with moderate and severe conditions. It is available under the Brand name Olumiant®, is an oral bio-available ATP competitive kinase inhibitor works selectively, potently and reversibly by inhibiting the JAK1 and JAK2 kinases. The JAK kinase is formerly associated with inflammation and immune function that causes rheumatoid arthritis. Baricitinib is thus potentially anti-inflammatory, immune-modulating and anti-neoplastic activities.[1-7,10]

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The IUPAC name of Baricitinib is 2-[1-Ethylsulfonyl-3-[4- (7H-pyrrolo [2,3-d] pyrimidin-4-yl) pyrazol-1-yl] azetidin-3-yl] acetonitrile as shown in figure 1.[8] Baricitinib has the chemical formula $C_{16}H_{17}N_7O_2S$ and molecular weight of 371.42g/mol. [9]

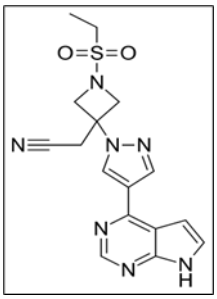

Figure 1: Structure of Baricitinib

The literature survey reveals that HPLC methods reported for the determination of Baricitinib. did not mention properly the composition of the mobile phase and its pH modifier. Hence an attempt has been made to develop a new HPLC method which is simple, rapid, reproducible, and economic for estimation of Baricitinib in tablet dosage form by HPLC.

2. Materials and Methods:

a. Chemicals and reagents:

Baricitinib working standard was procured from Central Drug Testing Laboratory (CDTL), Mumbai Central, Mumbai, with claimed potency [99.9%]. OLUMINANT® (4 mg), Baricitinib tablets were received as a gift sample from Assistant Drugs Controller Office, Air Cargo, Mumbai.

HPLC grade acetonitrile was procured from Rankem Laboratory Chemicals, sodium phosphate dibasic from Qualigens and sodium phosphate orthophosphate from Molychem. Water- milli-Q Grade were used for the analysis. All the chemicals were of analytical grade.

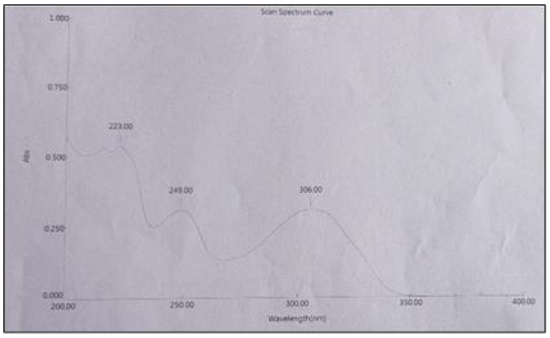

Figure 2: UV Spectrum of Baricitinib

b. Instrumentation:

- The chromatographic analysis was carried out using Eurosphere C18 column with 250mm x 4.6mm i.d.5µ particle size column.
- Thermo Scientific Dionex ultimate 3000 was applied,as a chromatographic method where a sample mixture or analyte is separated by a column with packing material at the high-pressure using pump with solvent passing through it.
- Thermo scientific Dionex ultimate 3000 is associated using software data system 7.2.6 with LC instrument control attached to the UV Detector.
- UV WinLab software was used for all the spectrophotometric measurements.

c. Selection of solvent (diluent):

Based on the solubility and chemical nature of Baricitinib, the mixture of Phosphate buffer (pH 6.5) and Acetonitrile (50:50) was selected as a diluent for the preparation of standard and sample solutions.

d. Selection of wavelength:

10 mg of Baricitinib was transferred to the 100ml volumetric flask and the volume was made up to the mark with diluent (100ppm). From that stock solution pipetted out 1ml into 10ml volumetric flask and volume was made up to the mark with diluent (10ppm). The solution was scanned in the range of 200 – 400 nm. The maximum absorbance was observed at 306 nm. Figure 2 shows the UV spectrum of Baricitinib.

e. Preparation of standard drug solution:

10 mg of Baricitinib standard drug was weighed accurately and transferred in 100ml of volumetric flask and dissolved by sonication with sufficient amount of diluent. The volume was made up to mark (100 µg/ ml). 2ml was pipetted out from the above stock solution and was diluted up to 10ml with diluent (20 µg/ ml).

f. preparation of sample solution:

A quantity of the powdered tablet, equivalent to 4 mg of Baricitinib was weighed and dissolved in sufficient amount of diluent, the volume was made up to 50ml with diluent (80µg/ ml). 2.5ml from the above solution

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was pipetted out in a 10ml volumetric flask and the volume was made up with diluent (20µg/ ml).

g. Method optimization:

The chemical structure of the Baricitinib shows that the drug is a basic and polar in nature. Hence, the molecule is selected according to its corresponding nature and its affinity towards the mobile phase. Initial trials were started with 0.1% trifluoroacetic acid (TFA) and acetonitrile with 50:50 ratio on BDS C18 column inersil but proper peak shape was not obtained. Further trials were done on PERKIN by using different mobile phase; phosphate buffer 6.5: acetonitrile at ratio 75:25 with zorbax eclipse column C18, here theoretical plates were appeared to be very less.The final method optimized after performing trials of mobile phase as phosphate buffer 6.5 and acetonitrile at the ratio of 70:30 with column EUROSPHERE 25cm × 4.6 mm & 5 µ, the Column oven temperature keeping at 40 ºC, the flow rate at 1.5ml/min gives a good and proper peak shape which comes to be at retention time of 4.9 minutes. The UV detected wavelength was set at 306 nm and system suitability test results were obtained as per limits. Figure 3 and 4 shows the optimized chromatograms of the Baricitinib standard and sample drug solution respectively. Optimized chromatographic condition of the drug were as shown in Table 1,

Table no. 1: Optimized chromatographic condition of the drug (Baricitinib)

Parameters	**Chromatographic condition**
HPLC System	Thermo ultimate 3000
Column	Eurosphere 25cm × 4.6 mm &5 µ
Mobile phase	Phosphate buffer 6.5: Acetonitrile (70:30)
Diluent	Phosphate buffer 6.5: Acetonitrile (50:50)
Standard solution concentration	Baricitinib stock solution (20ppm)
Flow rate	1.5ml/min.
Run time	10 min.
Wavelength	306 nm
Injection volume	20 µl
Temperature	40 ^{0}C
Retention time	4.9 minutes.
Detector	UV-VIS

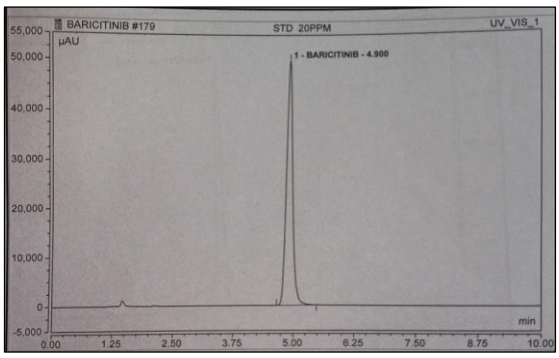

Figure 3: Chromatogram of Baricitinib standard solution

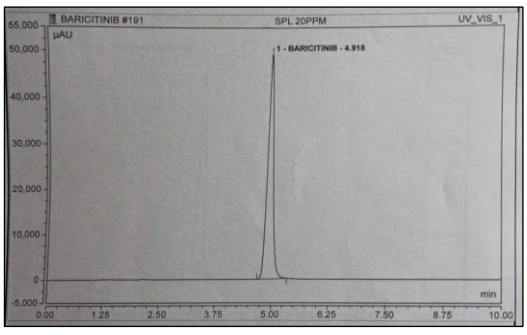

Figure 4: Chromatogram of Baricitinib sample solution

3. Validation Of Method

Validation of developed method on HPLC was done as per ICH Q2 (R1) guidelines with respect to various parameters such as specificity, system suitability, precision, linearity, accuracy, assay and robustness. Results of different tests were compared with these standard guidelines of ICH Q2 (R1) to get accurate results.

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a. Specificity:

1. The specificity was done on blank; standard & sample solutions and were studied accordingly. The running of blank chromatogram shows that the peak of blank does not hinder the standard & sample solution chromatogram and their results.
2. The peak purity of Baricitinib in tablet dosage form found within the limit which proved that there was no interference of the blank peaks and excipient peaks at the retention time of Baricitinib as shown in Figure 5, 6, and 7.

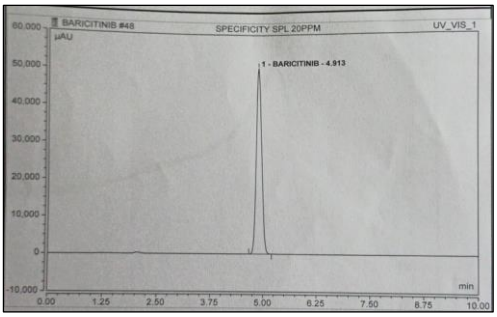

Figure 6. Chromatogram of Baricitinib sample solution

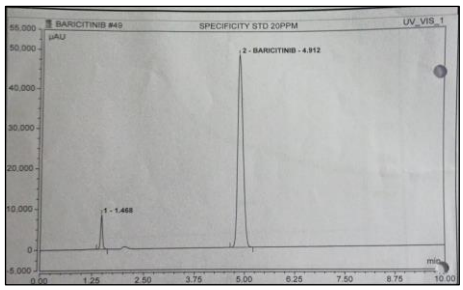

Figure 5. Chromatogram of Baricitinib standard solution

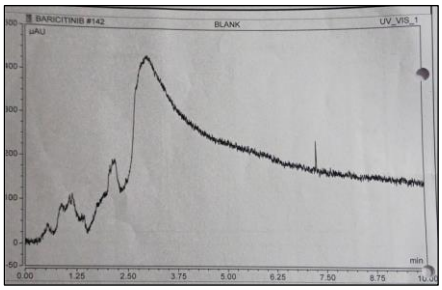

Figure 7: Chromatogram of blank solution

b. Linearity:

1. From the standard solution of Baricitinib, aliquots were prepared in the concentration range of 10-75ppm and run through the HPLC. A graph was constructed by plotting concentration vs. area obtained from the response reveals the linearity of the solution as shown in figure 8 as per table 2.
2. The linear calibration plot was constructed by analysing the concentrations over the selected range. The response for the drug was linear in the concentration range between 10-75 µg/ml. Table no.2 gives the linearity data of Baricitinib.

Table no.2: Linearity data of Baricitinib

Linearity level	Concentration (ug/ml)	Peak Area
1	5	1859.56
2	10	3746.63
3	15	5631.72
4	20	7346.88
5	25	9144.16
6	30	10930.84
7	40	14557.18
8	50	18574.02

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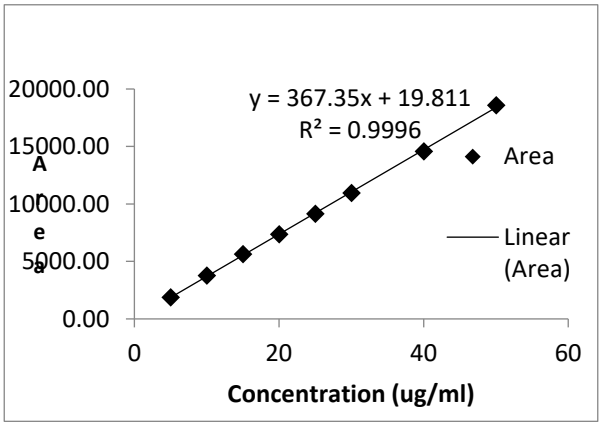

Figure 8: Calibration curve of Baricitinib

c. Accuracy (Standard Addition method):

Accuracy refers to the closeness of the measured value of a quantity corresponds to its "true" value. In that accepted either true value or an accepted reference value and the estimated value. Accuracy was carried out by standard addition method where standard and sample was added and by checking the percent recovery by adding it in the percentage of 110%, 120% & 130% as shown in table 3. In this test, the percent recovery was evaluated by notifying the mean recovery, Standard Deviation (S.D), Percent Relative Standard Deviation (%R.S.D). Accuracy data of Baricitinib is shown in the table no.3.

Table no.3: Accuracy of standard solution of Baricitinib

% LEVEL	Amount Spiked (ppm)	Amount Recovered (mg/tab)	%Recovery	Mean % Recovery	SD	% RSD
100	20.00	3.99	99.76	100.28	0.5251	0.5236
100	20.00	4.01	100.81			
100	20.00	4.03	100.27			
110	22.00	4.48	101.88	101.88	0.3755	0.3685
110	22.00	4.46	101.51			
110	22.00	4.50	102.26			
120	24.00	4.90	102.15	102.28	0.2861	0.2798
120	24.00	4.92	102.61			
120	24.00	4.90	102.08			
130	26.00	5.24	100.79	101.46	0.5981	0.5895
130	26.00	5.28	101.64			
130	26.00	5.30	101.94			

d. Precision:

Precision expresses the degree of reproducibility of responses of repeated measurements. The more responses and the smaller the error will give the best precision. 6 injections of sample and standard solution were run through HPLC to check precision of method.

e. System precision:

In system precision, six injections of standard solution (20ppm) were given and their responses were obtained. Further from standard solution, precision was determined. From the below table no.4 %RSD was found to be NMT 2.0% concluded to be within the acceptable limits as shown in the table no.4.

Table no.4: System precision of Baricitinib standard solution (20ppm)

System Precision (Standard)	
Injection No.	Area at 306 nm
1	7429.20
2	7426.80
3	7417.50

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4	7404.50
5	7417.40
6	7416.59
Average	7418.67
SD	8.78
%RSD (Limit NMT 2%)	0.12

f. Method precision:

In this, sample solution of 20ppm was prepared and 6 injections of the sample was studied as shown in table no.5 depicting Relative Standard Deviation (% RSD) to be not more than 2.0% as shown in the table no.5

Table no. 5: Method Precision of sample solution (20ppm)

Method Precision (sample)	
Injection No.	% Assay
1	99.52
2	99.35
3	99.77
4	99.06
5	101.42
6	100.22
Average	99.89
SD	0.85
% RSD (Limit NMT 2%)	0.85

Intra-day precision: The study was carried out by preparing the fresh solution and injecting in different time intervals i.e., at 10AM, 1.00PM and 4.00PM.The evaluation results of mean, S.D. and % R.S.D. are given in Table no.6.

Table no. 6: Intra-day precision of Baricitnib sample and standard solution

Intraday Precision			
Sr. No.	10:00 AM	01:00 PM	4:00PM
	% assay	% assay	% assay
1	96.86	100.94	101.40
2	96.36	101.03	101.31
3	96.45	100.82	101.37
4	96.43	100.91	102.62
5	95.74	100.91	102.59
Mean	96.36	100.91	101.85
SD	0.4020098	0.076359	0.68179
RSD	0.4171592	0.075664	0.669348
Limit	Not more than 2.0%	Not more than 2.0%	Not more than 2.0%

Inter-day/ intermediate day precision:

A freshly prepared standard solution was injected on two different days and by the different analysts to assess its reproducibility. The average percentage mean, standard deviation (S.D), and percentage relative standard deviation (%RSD) were calculated as follows (Table no.7):

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Table no.7: Inter-day precision

Inter-day Precision		
Sr. no.	Day 1	Day 2
	Analyst A	Analyst B
1	101.54	100.91
2	101.69	99.93
3	101.60	101.09
4	101.78	100.11
5	101.70	100.96
Mean	101.66363	100.6000
SD	0.0934637	0.537708
%RSD	0.0919343	0.534501
Limit	NMT 2.0%	NMT 2.0%

ROBUSTNESS:

The terms robustness and ruggedness refer to the ability of an analytical method to remain unaffected by small variations in the method parameters (mobile phase composition, column temperature, etc.) and influential environmental factors (room temperature, air humidity, etc.) and characterize its reliability during normal usage.

Deliberated changes were made in temperature, flow rate , wavelength, and mobile phase in the estimation of Baricitinib solution. Table no.8 gives the resulted data for the robustness of Baricitinib. Findings indicates the Reliability of the method. The mean, S.D., and %R.S.D. were detected.

Table no.8: Robustness of Baricitinib

Parameters	Change in parameter	% Estimation	Mean	SD	% RSD	Limit
Wavelength	304	101.90	101.26	1.10	1.09	NMT 2.0%
	306	99.99				
	308	101.89				
Flow rate	1.3	101.79	101.64	1.06	1.04	NMT 2.0%
	1.5	100.52				
	1.7	102.62				
Temperature	38	100.12	100.52	0.71	0.71	NMT 2.0%
	40	100.10				
	42	101.34				
Mobile Phase Ratio	35 65	100.63	100.70	0.96	0.96	NMT 2.0%
	30 70	99.78				
	25 75	101.70				

System Suitability Test (SST):

As system suitability test was an integral part of chromatographic methods development and it was carried under ICH (Q2) guidelines. After the HPLC method was optimized it was required to check their suitability and stability of that optimized method.

The injections of blank (1injection) and standard Baricitinib (6 injection replicates) were given at a working concentration of 20ppm. The chromatograms obtained by which peak area, retention time, theoretical plates and tailing factor of standard solution were determined and are mentioned as follows: (Table no.9)

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Table no.9: system suitability data for baricinintib

Sr. No.	Peak Area	Retention Time	Theoretical Plates	Tailing Factor
1	7495.495	4.900	7300	1.21
2	7487.311	4.902	7283	1.22
3	7477.117	4.900	7342	1.21
4	7391.938	4.897	7335	1.21
5	7500.512	4.900	7304	1.23
6	7500.160	4.907	7337	1.22
Average	7475.422	4.901	7317	1.22
S.D.	41.85	0.003	24.3427	0.01
%R.S.D.	0.56	0.068	0.3327	0.67
Limits	NMT 2.0%	NMT 1.0%	NLT 2000	NMT 2.0%

ASSAY

Six sample of Baricitinib of 20ppm were prepared and injected into the chromatographic system. The mean, standard deviation, and % RSD of the assay percentage of Baricitinib in the sample solution were calculated as shown in the table no.10.

Table no.10: Assay of Baricitinib

Sr. No.	Sample Weight (eq. to 4 mg)	Area of sample at 306nm	Assay
1	1 Tablet	7478.853	99.52
2	1 Tablet	7476.114	99.35
3	1 Tablet	7463.193	99.77
4	1 Tablet	7505.899	99.06
5	1 Tablet	7433.288	101.42
6	1 Tablet	7459.074	100.22
Average		7469.404	99.89
SD		24.15	0.85
%RSD (Limit NMT 2%)		0.32	0.85

4. Results and Discussion

- The developed method is a reproducible and simple method developed for the determination of Baricitinib tablet dosage form in which results obtained are as per their appropriate chromatographic conditions. Column used were as per the nature of the molecule, packing material used and structure of the molecule, dimension of the column, the composition, flow rate of mobile phase, the wavelength of detection, and injection volume.
- After optimizing the method as per the criteria, started with validation parameters. In that system suitability, assay, precision, etc. tests were performed. System suitability was performed in which tailing factor, theoretical plates, and retention time were determined and are found as per the specification of ICH guidelines.
- Linearity tests were performed in which different concentration sample vs. area performed and result obtained as y =367.35+19.811 and regression coefficient (r^2) of 0.996 which is within specifications. Precision tests were performed in which inter day, intraday, system precision, and method precision was performed as % R.S.D. not more than 2.0% in any of the test.
- Accuracy tests were performed in which closeness of the value and % mean recovery test performed in which % recovery is 99.760 and %R.S.D is not more than 2.0%. Assay tests were performed in which the content of the sample of Baricitinib is determined, in that % assay found was in between

99% to 102% & relative standard deviation (%R.S.D) was not more than 2.0%.

- By deliberately changing the parameters such as wavelength, flow & temperature robustness test is performed, the reliability of the method was concluded and % R.S.D. was not more than 2.0%. Specificity was performed in which sample, standard, blank chromatogram determined which indicating the other above peak is not hindering the actual peak of sample and standard.

5. Conclusion

The developed HPLC method is simple, specific, accurate, and precise for Baricitinib in a tablet dosage form. It was successfully validated in terms of linearity, accuracy, precision, specificity, and robustness in accordance with ICH guidelines. Also, the developed method is better than earlier published articles with respect to superior system suitability parameter such as theoretical plates, tailing factor. Thus, the described method is suitable for routine analysis and quality control of Baricitinib in bulk and tablet dosage forms.

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References

[1] Seelam Mohan, Natahani Srinivasarao, Lakshmi K. Development and validation of a stability indicating related susbstances of Baricitinib by RP-HPLC and its degradation. International Journal of Management and Humanities 2019. 4:4-9.

[2] Majithia Vikas, Geraci Stephen. Rheumatoid arthritis: diagnosis and management. The American Journal of Medicine 2007. 120(11):936-9.

[3] Fleischmann Roy, Alam Jahangir, Arora Vipin, Bradley John, Schlichting Douglas, Muram David, Smolen Josef. Safety and efficacy of baricitinib in elderly patients with rheumatoid arthritis. Rheumatic & Musculoskeletal diseases 2017.3(2):1-5.

[4] https://go.drugbank.com/drugs/DB11817

[5] National Center for Biotechnology Information (2021). PubChem Compound Summary for CID Retrieved June 1 2021.

[6] From https://pubchem.ncbi.nlm.nih.gov/compound/Baricitinib.

[7] International Conference on Harmonization (ICH), Q2 (R1) (Guidelines for validation of analytical procedures: text and methodology), (Accessed on January 15, 2019). http://www.ich.org/products/guidelinesquality/article/qualityguidelines.html

[8] Al-Salama Zaina, Scott Lesley. Baricitinib: A Review in rheumatoid arthritis. Adis Drug Evaluation 2018. 78:761-772.

[9] Mannurkar M.M. Hamrapurkar P.D. Development and validation of RP-HPLC method for Baricitinib using Quality by design approach and its application to stability studies. International Journal of Quality Assurance 2020. 12(1):40-47.

[10] Illendula Santhosh, Peddaboina Shiva Prasad. Analytical method development and validation of RP-HPLC for the quantitative determination of Baricitinib in pure substances and marketed formulation. International Journal of Pharmacy and Biological Sciences 2022. 12(3):108-114.

[11] Markham Anthony. Baricitinib: First Global Approval. Adisinsight Report 2017. 77:697-704.