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Development and Validation of UV Spectroscopic Method for Estimation of Baricitinib.

Santosh V. Gandhi*, Barkha G. Kapoor

AISSMS College of Pharmacy, Savitribai Phule Pune University, Pune, India-411001

ABSTRACT

A simple, sensitive and reproducible spectrophotometric method for the analysis of Baricitinib in pure form and in its dosage form has been developed. Baricitinib is a synthetic antineoplastic and immunomodulating drug. Baricitinib is a selective and reversible Janus kinase 1 (JAK1) and 2 (JAK2) inhibitor. Janus kinases belong to the tyrosine protein kinase family and play an important role in the proinflammatory pathway signalling that is frequently over-activated in autoimmune disorders such as rheumatoid arthritis. Developed method obeyed beer's law in a concentration range of 10-60 µg/ml with a correlation coefficient (R^2) of 0.993. Quantification was carried out at 250 nm. Percentage assay of Baricitinib was found to be close to 100 %. The results of analysis have been validated statistically and recovery studies confirmed the accuracy of the proposed method.

Keywords: Spectrophotometric method, Baricitinib, Antineoplastic, immunomodulating, Beer's law.

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***Address for Correspondence:**

Dr. Santosh V. Gandhi, Professor, AISSMS College of Pharmacy, Savitribai Phule Pune University, Pune, India-411001

INTRODUCTION:

Baricitinib is chemically, 2-[1-(ethanesulfonyl)-3-(4-{7H-pyrrolo[2,3-d]pyrimidin-4-yl}-1H-pyrazol-1-yl)azetidin-3-yl]acetonitrile. It is used for the treatment of moderate to severe active rheumatoid arthritis in adult patients who have responded inadequately to, or who are intolerant to one or more disease-modifying anti-rheumatic drugs as monotherapy or in combination with methotrexate. Upon administration, baricitinib binds to JAK1/2, which inhibits 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 and may prevent an inflammatory response. In addition, baricitinib may induce apoptosis and reduce proliferation of JAK1/2-expressing tumor cells. JAK kinases are intracellular enzymes involved in cytokine signaling, inflammation, immune function and hematopoiesis; they are also upregulated and mutated in various tumor cell types. In February 2017, Baricitinib was approved for use in the EU as a second-line oral therapy for moderate to severe active rheumatoid arthritis in adults, either alone or in combination with methotrexate. It is marketed under the trade name Olumiant. [1-3]. The structure of Baricitinib is given in Fig 1.

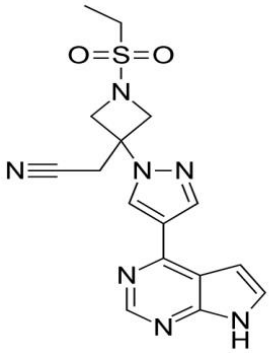

Fig 1: Structure of Baricitinib

As per the literature survey the data shows that simultaneous quantification of Baricitinib and Methotrexate in rat plasma by LC-MS/MS is reported [4]. No spectroscopic method has been reported for estimation of baricitinib in bulk or dosage form. The present manuscript describes simple and sensitive spectroscopic procedure for the determination of baricitinib in accordance with International Conference on Harmonisation Guidelines.[5]

MATERIALS AND METHODS:

CHEMICALS AND REAGENTS:

Baricitinib bulk powder was kindly gifted by Bulat Pharmaceutical Pvt. Ltd. India. Methanol AR grade was purchased from S.D. Fine Chemical Laboratories, Mumbai and DMSO AR grade was purchased from Loba Chemie Pvt. Ltd., Mumbai.

Preparation of standard stock solution:

Standard stock solution of drug was prepared by dissolving 10 mg of drug in 1 ml of DMSO (as the drug is sparingly soluble in methanol) and future volume was made up with methanol till 10 ml to get concentration of 1000 µg/ml. From this solution 1 ml was taken in 10 ml volumetric flask and volume was made up with methanol to get concentration of solution 100 µg/ml. Further 1 ml of this solution was diluted to 10 ml with methanol to get concentration of solution 10 µg/ml.

Selection of detection wavelength:

From the standard stock solution (1000 µg/ml)further dilutions were made using methanol and scanned over the range of 220-375 nm against methanol as blank and the spectra was obtained. It was observed that the drug showed linear, stable and considerable absorbance at 250 nm. Representative UV spectrum of Baricitinib is shown in Fig. 2

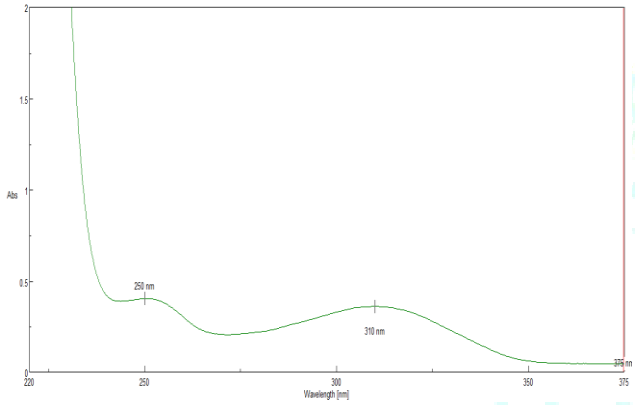

Figure 2: UV Spectrum of Baricitinib (10 µg/ml)

In house preparation of formulation (For Assay)

Formulation for Baricitinib was prepared by geometric mixing of commonly used excipients for tablet formulation with drug. All ingredients were accurately weighed and geometrically mixed. List of the ingredients is shown in Table 1.

Table 1: List of ingredients for in house preparation of Formulation

Name of Ingredient	Quantity Taken (for 100mg)
Baricitinib	2 mg
Microcrystalline	28 mg
Mannitol	70 mg

VALIDATION OF THE PROPOSED METHOD:

The method was validated with respect to linearity, accuracy, precision, limit of detection and limit of quantification according to the ICH guidelines [5]

Linearity:

Standard stock solution of drug was prepared by dissolving 10 mg of drug in 1 ml of DMSO (as the drug is sparingly soluble in methanol) and future volume was made up with methanol till 10 ml to get concentration of 1000 µg/ml. Further 1 ml was taken and diluted to 10 ml with methanol in volumetric flask to get 100 µg/ml. This solution was further diluted with methanol to get range of solution containing different concentrations 10-60 µg/ml. Absorbance was noted at λ max 250 nm. The equation of calibration curve by UV- Spectroscopy was found to be y=0.031 x+0.080 with R^2 = 0.993. The absorbance of drug was plotted against the corresponding concentrations to obtain the calibration curve as shown in Fig. 3

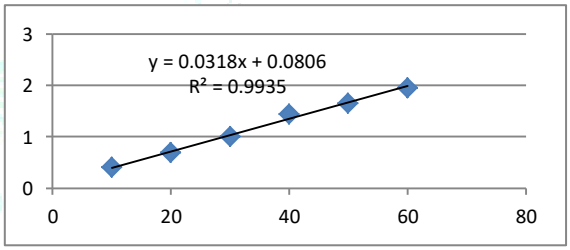

Fig 3: Linearity curve of Baricitinib by UV- Spectroscopy (10-60 µg/ml).

PRECISION:

The intraday and interday precision of the proposed method were performed by analyzing the corresponding responses 3 times on the same day and on 3 different days for 3 different concentrations of standard solutions of Baricitinib (20, 30 and 50 µg/ml) without changing the parameters for the method. The results were reported in terms of relative standard deviation (% RSD). The results obtained for intra-day and inter-day variations by UV-Spectroscopy are shown in Table 2 and Table 3, respectively.

Table 2: Intra-day variation studies data for Baricitinib

Replicates	Conc. (µg/ml)		
	20	30	50
	Absorbance		
1	0.715	1.024	1.628
2	0.709	1.017	1.638
3	0.708	1.002	1.649
Average	101.710	100.455	100.542
SD	0.643	1.235	0.678
%RSD	0.632	1.229	0.674

Table 3: Inter-day variation studies data for Baricitinib.

Replicates	Conc. (µg/ml)		
	20	30	50
	Absorbance		
1	0.711	1.049	1.627
2	0.710	1.027	1.648
3	0.709	1.011	1.659
Average	101.590	102.008	100.948
SD	0.113	2.045	1.056
%RSD	0.111	2.005	1.046

Limit of Detection (LOD) and Limit of Quantification (LOQ):

From the linearity data the LOD and LOQ as calculated, using the formula LOD = 3.3 σ/S and LOQ = 10 σ/S, where σ = standard deviation of the y intercept of linearity equations and S =slope of the calibration curve of the analyte. The LOD and LOQ by UV-Spectroscopy were found to be 0.250 µg/ml and 0.756 µg/ml, respectively.

Assay Procedure:

In house drug formulation was prepared. Quantity of formulation equivalent to 10 mg of drug was weighed and transferred to 10 ml volumetric flask, dissolved in 1 ml of DMSO and volume made to 10 ml with methanol. The solution was filtered through Whatmann filter paper and it is suitably diluted with methanol to obtain the concentration of 10 µg/ml. Procedure was repeated for six times. Results obtained are shown in Table 4.

Table 4: Assay of inhouse prepared formulation.

SN	Absorbance	Amount Recovered (µg/ml)	% Recovery
1	0.390	10.003	100.026
2	0.392	10.065	100.655
3	0.390	10.002	100.019
4	0.390	10.004	100.039
5	0.390	10.005	100.055
6	0.391	10.033	100.329
Mean	0.391	10.019	100.187
SD	0.001	0.026	0.258
%RSD	0.205	0.257	0.257

Accuracy:

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50 %, 100 % and 150 %. The drug concentrations and % recovery was determined from linear equation. Results obtained are shown in Table 5.

Table 5: Accuracy of Baricitinib

Level	Conc. of Sample solution (µg/ml)	Conc. of Standard solution spiked (µg/ml)	Absorbance	Amount recovered (µg/ml)	% recovery ± % RSD
50%	10	5	0.544	14.958	99.741 ± 0.376
			0.542	14.906	
			0.546	15.019	
100%	10	10	0.711	20.339	100.726 ± 0.833
			0.702	20.058	
			0.701	20.039	
150%	10	15	0.870	25.494	100.586 ± 1.514
			0.861	25.206	
			0.877	24.739	

Robustness:

Robustness of the method was determined by carrying out the analysis under conditions during which detection wavelength (± 2 nm) was altered and the effect on the absorbance was noted. The method was found to be robust. The result of the robustnesss for Baricitinib is shown in Table 6

Tablet 6: Robustness Study for Baricitinib

% RSD Found For Robustness Study (Absorbance of 20 µg/ml)		
DETECTION WAVELENGTH (± 2 nm)		
248	**250**	**252**
0.636	0.648	0.635

RESULT AND DISCUSSION:

In the proposed method, Baricitinib showed absorption maxima at 250 nm. The calibration curve was found to be linear in the concentration range of 10-60 µg/ml. Accuracy was determined by calculating the recovery. Precision was calculated as intra and inter day variation (% RSD) for Baricitinib. LOD value for Baricitinib was found to be 0.250 µg/ml and LOQ value is and 0.756 µg/ml respectively. The method was successfully used to determine the amounts of Baricitiib present in formulation.

Table 7: Summary of Validation Parameters

Sr. No.	Validation parameters	Baricitinib
1.	Linearity equation R^2 Range	y = 0.031 x + 0.080 R^2= 0.993 10-60 µg/ml
2.	Precision	(%RSD)
	Intraday	0.632 – 1.229 %
	Inter-day	0.111 – 2.005 %
3.	Assay	100.187 %
4.	Accuracy	Mean ± RSD
	50 %	99.741 ± 0.376
	100 %	100.726 ± 0.833
	150 %	100.586 ± 1.514
5.	Limit of detection	0.250 µg/ml
6.	Limit of quantitation	0.756 µg/ml
7.	Specificity	Specific
8.	Robustness	Robust

CONCLUSION:

The method proposed in the above study was found to be simple, specific, economic, precise and rapid for the determination of Baricitinib in bulk as well as in its dosage form. Being economic and precise, the developed method may conveniently adopted as an alternative method for the routine analysis of the Baricitinib in bulk and pharmaceutical dosage form.

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