Stability Indicating Method Development and Validation of Baricitinib in Bulk and Formulation Using UV Spectroscopy

Khagga Bhavya Sri[1]*, Mohammad Saba Fatima[2], Mogili Sumakanth[3]

[1]Department of Pharmaceutical Analysis, HOD of Department of Pharmaceutical Analysis, RBVRR Women's College of Pharmacy, Hyderabad, Telangana, India.
[2]Department of Pharmaceutical Analysis, RBVRR Women's College of Pharmacy, Hyderabad, Telangana, India.
[3]Department of Pharmaceutical Chemistry, Principal at RBVRR Women's College of Pharmacy, Hyderabad, Telangana, India.

ABSTRACT

Baricitinib is a novel drug that was approved for use in treating moderate to severe rheumatoid arthritis, and severe alopecia areata. It also became the first immunomodulatory drug to get FDA approval in 2022 for treating COVID-19 in hospitalized adults requiring supplemental oxygen. It is a selective JAK inhibitor that binds reversibly and inhibits Janus Kinase 1 and Janus Kinase 2. The present study aimed to develop a method to demonstrate the stability and validation of baritinib in bulk and formulation using UV spectroscopy. A simple, precise, and economical method has been developed for the estimation of baricitinib in bulk and formulation. The UV spectroscopic method was developed using DMF (Dimethylformamide) and distilled water as the diluent. The standard stock solution for the UV spectroscopic method was prepared by dissolving 10 mg drug in 1 mL DMF and later made up with distilled water. The subsequent dilutions were prepared using distilled water and the dilutions were quantified at 309 nm. The method was validated according to the ICH guidelines Q2 R (2). Linearity was found to be in the concentration range of 10-100 µg/mL with a good correlation coefficient (r^2) of 0.999. The precision was found to be within the acceptable limits (%RSD < 2.0). Therefore, the method developed is sensitive, reproducible, and precise, and can be used as the routine quality control test for baricitinib.

Key Words: *Baricitinib, COVID-19, JAK inhibitor, UV spectroscopic method*

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INTRODUCTION

Eli/Lilly Company developed baricitinib [Olumiant®] as a selective, reversible Janus kinase inhibitor to treat arthritis and dermatitis [1]. IUPAC name of baricitinib is 2-[1-[Ethylsulfonyl]-3-[4-7H-pyrrolo[2,3-d]pyrimidin-4-yl-1H-pyrazol-1-yl]azetidin-3-yl] acetonitrile which is an immunomodulatory drug [2, 3] with anti-inflammatory properties [4]. **Figure 1** shows the structure of baricitinib. For individuals with severe or moderate active rheumatoid arthritis, baricitinib was authorized for use in the EU in February 2017 [5]. It is used to treat moderate to severely active rheumatoid arthritis in adult patients who have not responded well to or are intolerant to one or more disease-modifying anti-rheumatic drugs as a monotherapy or in combination with methotrexate [6]. The FDA in the United States has approved its usage in conjunction with REMDS to treat hospitalized patients [7, 8]. After being administered, baricitinib binds to JAK1/2, preventing it from being activated, which then prevents the JAK signal transducers and the activators of the transcription (STAT) signaling pathway from being activated [9]. As a result, fewer inflammatory cytokines are produced, perhaps delaying the onset of inflammation. Baricitinib may also cause apoptosis and lessen the growth of tumor [10] cells that express JAK1/2 [11-13].

Corresponding author: Khagga Bhavya Sri
Address: Department of Pharmaceutical Analysis, HOD of Department of Pharmaceutical Analysis, RBVRR Women's College of Pharmacy, Hyderabad, Telangana, India.
E-mail: ✉ bhavya.khagga@gmail.com
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The literature survey reveals that there are few methods available like the two LC test techniques for determining its pharmacokinetics in rat plasma. The former employed LC/MS/MS to estimate baricitinib and methotrexate [14], whereas the latter used UPLC [15]. One UV-spectroscopic method for the determination of the drug's pure form and dosage form was developed which used DMSO as the diluent [9]. Furthermore, very few HPLC methods are available for the determination of baricitinib like the one which uses RPLC-Diode array detection system [9], the second method used Methanol: Phosphate buffer (45:55) as mobile phase with UV detector [16] and other employed QbD approach to in the method development [17]. The current method was developed to provide an easy and accurate UV spectroscopic method for determining baricitinib in bulk and in the formulation which is economical, precise, sensitive, and robust.

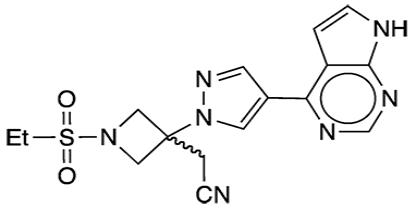

Figure 1. Structure of baricitinib

MATERIALS AND METHODS

Chemicals

Baricitinib API (Active Pharmaceutical Ingredient) was a gift from the pharmaceutical industry. A tablet formulation containing baricitinib 2 mg was bought from the local pharmacy. DMF (Dimethylformamide), and distilled water solvents were used.

Instrument

A double-beam UV-visible spectrophotometer-ELICO 210 was used in the method development and validation.

Selection of solvent

Baricitinib drug was checked for solubility in organic solvents DMSO, DMF, and Methanol. DMF was selected as the solvent to dissolve the drug during the method development.

Preparation of stock solutions

A stock solution of concentration 1000 µg/ml was prepared. It was done by first dissolving 10 mg of baricitinib pure drug in 1 ml of DMF and then made up to mark in the 10 ml Volumetric flask with distilled water. The working standard solution having a concentration of 100 µg/ml and the subsequent dilutions having required concentrations were prepared using distilled water as the diluent.

Preparation of calibration curve

The dilutions having different concentrations were prepared with distilled water. The λmax was found to be 309 nm when 10 µg/ml solution was kept and scanned in the UV-visible spectrophotometer against the blank distilled water. The calibration curve was plotted in the range of 10-100 µg/ml as the drug followed Beer-Lambert's law in that concentration range.

Quantification of sample

The 10 tablets containing baricitinib 4 mg were weighed and the average weight was calculated. The tablets were powdered and the equivalent weight of 10 mg pure drug was calculated from the average weight. The equivalent weight powder was taken in a 10 ml volumetric flask and 1 ml DMF was added to dissolve it, it was then made up to the mark with distilled water. The absorbance was checked at 309 nm and the actual concentration was calculated.

Method validation

Linearity and range: The linearity of baricitinib was determined from the calibration curve. The correlation coefficient (r^2) along with the equation (y = mx+c) was obtained by linear regression analysis. The range in which the drug shows linear response was also noted.

Precision: The repeatability of the method was determined by analyzing replicates of the same level of concentration 6 times i.e., at 40 µg/ml. The inter-day and intra-day precision was estimated by repeating the analysis of 40 µg/ml on different days by the same analyst and on the same day by different analysts respectively for the determination of the Intermediate precision. The precision is calculated by calculating the %RSD.

Accuracy: The accuracy of the method was confirmed by spiking the sample concentration with 50%, 100%, and 150% of standard drug concentration i.e., to the sample concentration of 20 µg/ml, 10 µg/ml, 20 µg/ml, and 30 µg/ml of baricitinib standard concentration was added and the recovery percentage was calculated. The spiking was done three times and the mean percentage recovery was then calculated.

Detection limit and the quantitation limit: The detection limit and the quantitation limit of the method developed were calculated from the calibration standards. The detection limit was calculated from the formula as per ICH guidelines Q2 R (2).

$$DL = \frac{3.3\,\sigma}{S} \quad (1)$$

$$QL = \frac{10\,\sigma}{S} \quad (2)$$

Where σ = Standard deviation of the response.
S = Slope of the calibration curve.

Robustness: The method developed was also validated with the robustness parameter. A small change in the method developed is assessed. Here the 40 µg/ml solution of the drug was scanned at + & - 1 nm of the λmax 309 nm.
Stability studies: The stability of baricitinib was determined by keeping the sample solution aside on a benchtop for 48 hrs at room temperature and absorbance was checked at different time intervals.

Forced degradation studies
Alkali degradation: 5 ml of the sample solution was transferred into two 10 ml volumetric flasks, to one flask 2 ml of 0.1 N NaOH was added and to another 2 ml of 1 N NaOH was added. It was kept aside for 10 mins and the absorbance was checked in a UV-visible spectrophotometer. The percent of drug degraded was calculated.
Acid degradation: 5 ml of the sample solution was transferred into two 10 ml volumetric flasks, to one flask 2 ml of 0.1 N HCl was added and to another 2 ml of 1 N HCl was added. It was kept aside for 15 mins and the absorbance was checked in a UV-visible spectrophotometer. The percent of drug degraded was calculated.
Peroxide degradation: 5 ml of the sample solution was transferred to a 10 ml volumetric flask 1 ml of 3% H2O2 was added and kept aside for 10 mins, the absorbance was checked, and the % drug degraded was calculated.
Thermal degradation: The sample drug solution was placed in a hot air oven at 45 ºC for 10 mins. Absorbance was checked and the % of drug degraded was calculated.
Photolytic degradation: The sample solution was kept in a UV chamber for 6 hrs. The sample was checked for absorbance in a UV-visible spectrophotometer.

Calculations
Assay calculations
The percentage purity of the marketed sample is calculated using the formula.

$$\%Assay = \frac{Absorbance\ of\ Sample}{Absorbance\ of\ Standard} \times \frac{Concentration\ of\ Standard}{Concentration\ of\ Sample} \times 100 \quad (3)$$

$$\%\text{Assay} = \frac{0.9095}{0.8074} \times \frac{40}{41.46} \times 100$$

% Assay = 1.044 × 0.96 × 100

% Assay = 100.22%

RESULTS AND DISCUSSION

The prepared dilutions of the pure baricitinib were scanned in the range of 200-400 nm. A sharp peak was obtained at λmax 309 nm as seen in **Figure 2**. The drug obeyed Beer-Lambert's law in the calibration curve range of 10-100 µg/ml where the linear response of the drug was recorded. The correlation coefficient was found to be 0.999 with the equation y = 0.0231x - 0.0399 as shown in the graph in **Figure 3**. The method developed showed good repeatability with % RSD = 0.267%, intermediate precision with intra-day and inter-day precision calculations was also performed and the results obtained had % RSD < 2.0 along with the robustness data with the % RSD = 0.164% and 0.136% at 308 and 310 nm, respectively as mentioned in **Table 1**. The method developed was robust. The recovery percentages were calculated for the accuracy of the method developed. The data related to the spiked studies for accuracy is shown in **Table 2** and the mean % recovery of the drug was calculated to be in the range of 97-99%. The detection limit (DL) and the quantitation limit (QL) calculated from the equations as mentioned in ICH Q2 R (2) guidelines were found to be 0.334 µg/mL was found to be 1.012 µg/mL, respectively. The stability data was obtained through the benchtop stability studies along with forced degradation studies with % drug degraded under different conditions are mentioned in **Table 3** and the graph in **Figure 4**.

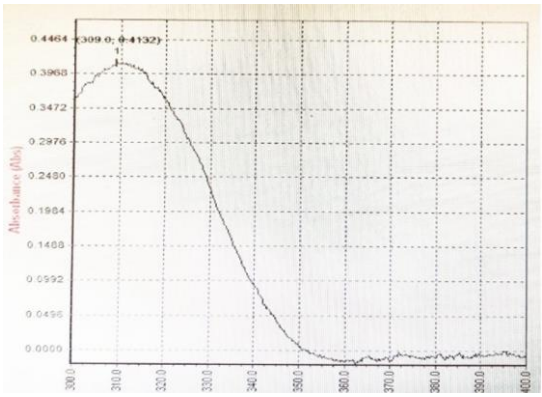

Figure 2. Determination of λmax of baricitinib pure drug

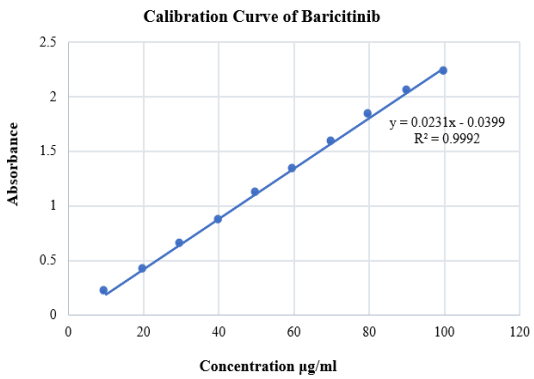

Figure 3. Calibration curve of baricitinib.

Table 1. % relative standard deviation of various validation parameters.

Validation Parameter	Repeatability	Intra-day Precision		Inter-day Precision		Robustness	
Condition	**309 nm**	**Analyst -1**	**Analyst-2**	**Day-1**	**Day-2**	**308 nm**	**310 nm**
% RSD	0.26756	0.26756	0.22101	0.26756	0.25157	0.16451	0.13626

Table 2. Recovery studies data of the method developed.

Percentage level	Absorbance	Recovery (%)	Recovery (Mean %)
50% **(20 ppm + 10 ppm)**	0.6004	96.99%	97.12%
	0.6011	97.10%	
	0.6013	97.14%	
100% **(20 ppm + 20 ppm)**	0.8217	99.49%	99.51%
	0.8221	99.53%	
	0.8219	99.51%	
150% **(20 ppm +30 ppm)**	1.0311	98.00%	98.04%
	1.0315	98.04%	
	1.0319	98.08%	

Table 3. Forced degradation studies data of baricitinib.

Type of degradation studies	Condition	Degraded (%)	Degradation (Mean %)
Benchtop (Stability study)	6 HRS	1%	1.5%
	24 HRS	1.50%	
	30 HRS	1.70%	
	48 HRS	1.8%	
Acid	0.1 N HCl	12.20%	14.28%
	1 N HCl	16.35%	
Alkali	0.1 N NaOH	16.50%	19.50%
	1 N NaOH	22.50%	
Peroxide	3% H_2O_2	11.20%	11.20%
Thermal	At 45 °C	9.80%	9.80%
Photolytic	UV Chamber	10.30%	10.30%

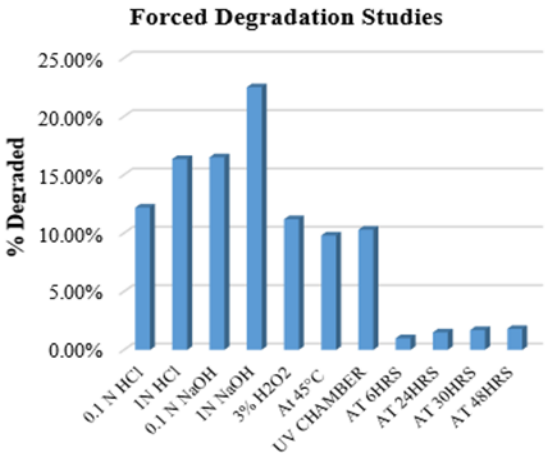

Figure 4. Forced degradation studies graph of baricitinib.

CONCLUSION

The measurement and quantification of baricitinib in bulk and the formulation were found to be simple, accurate, linear, robust, and quick using the suggested UV spectroscopic approach. The UV technique that was developed is simple and produces accurate results. Since the technology is both practical and affordable, it may be used for regular quality control examinations of the baricitinib dosage form.

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Ethics statement: None

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