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Development and Validation of a Stability-Indicating UV Spectroscopic Method for Baricitinib in Bulk and Formulation

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ABSTRACT

Baricitinib is a novel therapeutic agent recently approved for the treatment of moderate to severe rheumatoid arthritis, severe alopecia areata, and since 2022, COVID-19 in hospitalized adults requiring supplemental oxygen. This drug acts as a selective inhibitor of Janus kinase 1 and 2. This study aimed to establish and validate a stability-indicating UV spectroscopic method for the measurement of baricitinib in both bulk and formulated forms. A straightforward, precise, and cost-effective UV method was developed using dimethylformamide (DMF) and distilled water as the solvent. To prepare the standard stock solution, 10 mg of baricitinib was dissolved in 1 mL of DMF and then diluted with distilled water. Subsequent dilutions were prepared using distilled water and analyzed at a wavelength of 309 nm. The validation of this method was performed according to ICH Q2 R(2) guidelines. The method demonstrated linearity in the concentration range of 10-100 µg/mL with an excellent correlation coefficient (r^2 = 0.999). The precision results were within the acceptable range, and the % RSD was less than 2.0. Therefore, this developed method is reliable, sensitive, and reproducible, making it suitable for routine quality control analysis of baricitinib.

Keywords: UV spectroscopic method, Baricitinib, JAK inhibitor, COVID-19

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Introduction

Baricitinib [Olumiant®] is a selective Janus kinase (JAK) inhibitor developed by Eli/Lilly company to treat conditions such as arthritis and dermatitis [1]. The drug, with the IUPAC name 2-[1-[ethylsulfonyl]-3-[4-7H-pyrrolo[2,3-d]pyrimidin-4-yl-1H-pyrazol-1-yl]azetidin-3-yl] acetonitrile, functions as an immunomodulatory agent with anti-inflammatory properties [2-4]. A visual representation of baricitinib's structure is shown in **Figure 1**. Initially authorized by the EU in 2017 for treating moderate to severe active rheumatoid arthritis, baricitinib is used in adult patients who do not respond adequately or are intolerant to traditional disease-modifying anti-rheumatic drugs (DMARDs), either alone or in combination with methotrexate [5, 6]. In the U.S., the FDA approved it for use alongside REMDS in hospitalized COVID-19 patients [7, 8]. Baricitinib acts by binding to and inhibiting JAK1/2, which prevents activation of the JAK-STAT signaling pathway, ultimately reducing the production of inflammatory cytokines and alleviating inflammation [9]. Additionally, it may induce apoptosis and inhibit the growth of cancer cells expressing JAK1/2 [10-13].

A review of the existing literature reveals a few analytical techniques for measuring baricitinib. These include two LC-based methods for studying its pharmacokinetics in rat plasma, one of which uses LC-MS/MS to quantify baricitinib and methotrexate [14], and another employing UPLC [15]. One UV spectroscopic method has been

developed for analyzing baricitinib in pure and dosage forms, using DMSO as the solvent [9]. Moreover, several HPLC methods have been established, such as those utilizing an RPLC-diode array detection system [9], Methanol: phosphate buffer (45:55) as the mobile phase with UV detection [16], and a method developed using a quality-by-design (QbD) approach [17]. In this study, we propose an efficient, reliable, and cost-effective UV spectroscopic method to quantify baricitinib in both bulk and formulation forms, offering high precision, sensitivity, and robustness.

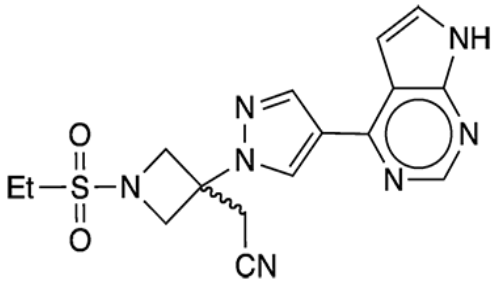

Figure 1. Structure of baricitinib

Materials and Methods

Chemicals

Baricitinib's active pharmaceutical ingredient (API) was graciously provided by a pharmaceutical company. A baricitinib tablet (2 mg) was purchased from a local pharmacy. The solvents used for the procedure included Dimethylformamide (DMF) and distilled water.

Instrument

The spectrophotometric analysis and method validation were performed using a double-beam UV-visible spectrophotometer (ELICO 210).

Solvent selection

To assess the solubility of baricitinib, the drug was tested in various solvents, including DMSO, DMF, and methanol. DMF was ultimately chosen as the ideal solvent for the drug during the method development process.

Preparation of stock solutions

To prepare a stock solution of baricitinib, 10 mg of the drug was dissolved in one ml of DMF. The solution was then diluted to a final volume of 10 ml using distilled water in a volumetric flask, resulting in a concentration of 1000 µg/ml. The working solution was prepared by further dilution, resulting in a concentration of 100 µg/ml, with distilled water serving as the diluent.

Development of calibration curve

For calibration, several different concentrations of the drug were prepared using distilled water. The λ_{max} of the drug was determined to be 309 nm, based on a 10 µg/ml solution scanned against a distilled water blank. A calibration curve was then plotted for the concentration range of 10-100 µg/ml, where the drug followed Beer-Lambert's law.

Quantification of the sample

To quantify baricitinib in the tablet form, ten tablets, each containing 4 mg of the drug, were weighed. The average weight was calculated, and the tablets were powdered. An equivalent weight of ten mg of pure baricitinib was then determined. This powdered drug was dissolved in one ml of DMF in a ten ml volumetric flask and diluted with distilled water. The absorbance was measured at 309 nm, and the concentration of the drug was calculated.

Validation of method

Linearity and range: Linearity was evaluated using the calibration curve, and the correlation coefficient (r^2) was calculated through linear regression analysis. The concentration range that exhibited linearity was also recorded.
Precision: To assess precision, the method was tested by analyzing 6 replicate samples at 40 µg/ml concentration. Precision was further examined for intra-day and inter-day variations by performing the same test across different days and different analysts, with results expressed as the relative standard deviation (% RSD).
Accuracy: The accuracy of the method was validated by spiking known concentrations (50, 100, and 150%) of the baricitinib standard to a 20 µg/ml sample. The recovery percentages were calculated after spiking the drug with concentrations of 10, 20, and 30 µg/ml. The experiment was performed 3 times, and the average recovery was determined.
Detection limit and quantitation limit: The calibration standards were used to determine both the detection and quantitation limits of the proposed method. The detection limit was derived using the formula specified in the ICH Q2 R (2) guidelines.

$$DL = (3.3\ \sigma)/S \quad (1)$$

$$QL = (10\ \sigma)/S \quad (2)$$

Where σ = standard deviation of the response, and S = slope of the calibration curve.

Robustness: The robustness of the developed method was evaluated by making small, intentional changes to the established procedure. Specifically, a 40 µg/ml concentration of the drug was scanned with a ± 1 nm variation around the λ_{max} of 309 nm to assess the method's stability under these slight modifications.
Stability Investigation: To examine the stability of baricitinib, a sample solution was left on a benchtop at room temperature for 48 hours, with absorbance measured at regular time intervals to track any changes over time.

Forced degradation studies
Alkaline conditions: For alkaline degradation testing, five ml of the sample solution was split into two 10 ml volumetric flasks. Each flask received 2 ml of either 0.1 N NaOH or 1 N NaOH. After allowing the mixture to sit for 10 minutes, the absorbance was recorded using a UV-visible spectrophotometer, and the degradation percentage of the drug was determined.
Acidic conditions: In this test, 5 ml of the sample solution was divided into two 10 ml volumetric flasks, with each receiving 2 ml of either 0.1 N HCl or 1 N HCl. After a 15-minute incubation period, absorbance readings were taken to evaluate the extent of degradation.
Peroxide-induced degradation: A 5 ml sample was transferred to a 10 ml volumetric flask, and 1 ml of 3% hydrogen peroxide was added. After a 10-minute reaction time, absorbance was measured to determine the percentage of degradation.
Thermal stress: The sample solution was heated in a hot air oven at 45 ºC for ten minutes. Afterward, absorbance readings were taken to assess the extent of degradation caused by thermal exposure.
Light-induced degradation: The sample solution was exposed to UV light for 6 hours, and absorbance was checked afterward to quantify any degradation resulting from light exposure.

Calculations
Assay calculation method
The purity percentage of the commercially available sample is determined by applying the formula:

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

$$\%\ \text{Assay} = \frac{0.9095}{0.8074} \times \frac{40}{41.46} \times 100$$
$$\%\ \text{Assay} = 1.044 \times 0.96 \times 100$$

% Assay = 100.22%

Results and Discussion

The dilutions of baricitinib were examined within the wavelength range of 200–400 nm, yielding a prominent peak at 309 nm **(Figure 2)**. This drug demonstrated adherence to Beer-Lambert's law across a concentration range of 10–100 µg/ml, with a clear linear correlation. The computed correlation coefficient was 0.999, and the derived equation was y = 0.0231x - 0.0399, as shown in **Figure 3**. The precision of the developed method was confirmed with a % RSD of 0.267%, while both intra-day and inter-day precision evaluations yielded results below the 2.0% RSD threshold. Additionally, the method exhibited excellent robustness with % RSD values of 0.164% and 0.136% at 308 nm and 310 nm, respectively, as outlined in **Table 1**. Accuracy was assessed via recovery studies, with the mean recovery percentage ranging between 97% and 99%, as detailed in **Table 2**. According to ICH Q2 R (2) guidelines, the detection and quantitation limits were found to be 0.334 µg/mL and 1.012 µg/mL, respectively. Furthermore, stability assessments, including benchtop and forced degradation tests, were conducted, and the degradation percentages under various conditions are presented in **Table 3** and **Figure 4**.

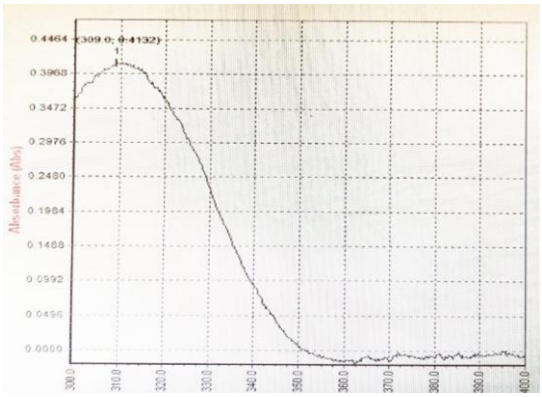

Figure 2. Determination of λ_{max} of baricitinib pure drug

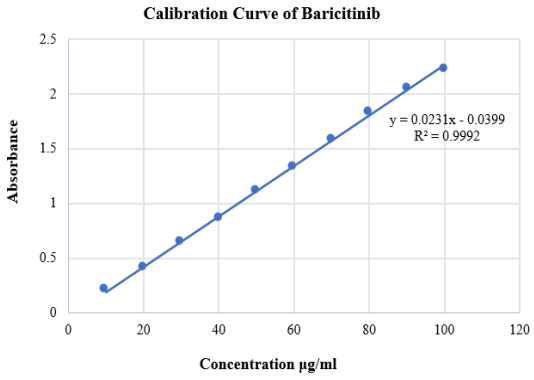

Figure 3. Calibration curve of baricitinib.

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%

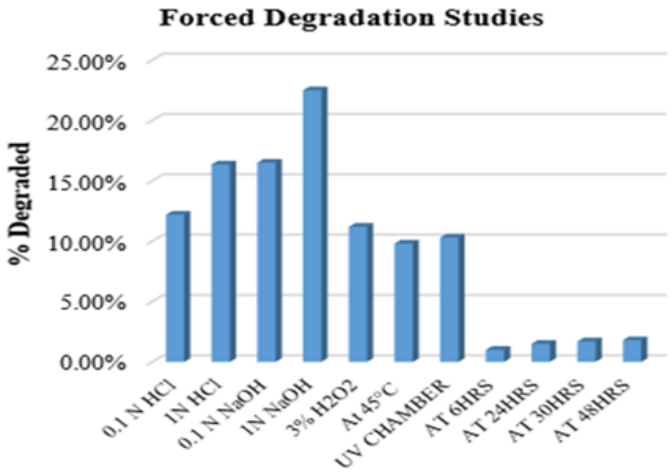

Figure 4. Forced degradation studies graph of baricitinib.

Conclusion

The UV spectroscopic method proposed for the analysis of baricitinib in both bulk and formulation was found to be effective, providing accurate, linear, and reproducible results with minimal time requirements. This method is not only straightforward but also delivers reliable outcomes. Due to its simplicity and cost-efficiency, it is ideal for frequent use in quality control testing of baricitinib formulations.

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Conflict of Interest: None

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Ethics Statement: None

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