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DEVELOPMENT AND VALIDATION OF NOVEL RP-HPLC METHOD FOR THE ESTIMATION OF BARICITINIB

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ABSTRACT:
A novel medication called Baricitinib was licensed to treat severe alopecia areata and moderate-to-severe rheumatoid arthritis. In 2022, it was also approved by the FDA as the first immunomodulatory medication for the treatment of COVID-19 in hospitalized people who needed extra oxygen. It is a reversible inhibitor of JAK that is selective for JAK1, and JAK2. A straightforward, accurate, cost-effective approach has been devised for the estimation of Baricitinib in both bulk and tablet formulation. Methanol was used as the diluent in the development of this HPLC technique. The stock solution was prepared using methanol, 10 mg of the pure drug was dissolved in 2-3 ml of methanol, which was then made up of the same. Methanol was used to prepare the dilutions that followed, and the dilutions were measured at 249nm. The approach was verified in compliance with ICH criteria Q2 R(1). The linearity was determined to be between 10µg/mL and 80µg/mL in concentration, with a correlation coefficient (r2) of 0.999. Accuracy was determined to be within allowable bounds (%RSD < 2.0). As a result, the technique created is accurate, repeatable, sensitive, and suitable for routine Baricitinib quality control examination.
Keywords: COVID-19, Baricitinib, JAK inhibitor, HPLC method.

INTRODUCTION:

Baricitinib is a selective, reversible Janus kinase inhibitor that was developed by the Eli/Lilly Company to treat dermatitis and arthritis (Taylor et al., 2017).Baricitinib's IUPAC name is 2-[1-[Ethylsulfonyl]4-[7H-pyrrolo[2,3-d] -3-[1-H-pyrazol-1-yl pyrimidin-4-yl]Zetidin-3-yl acetonitrile, a medication having anti-inflammatory and immunomodulatory effects (AlRuwaili et al., 2022, and Ukibayev et al., 2021). The structure of Baricitinib is depicted in Figure 1. In February 2017, the EU approved its usage for people with moderate to severe active rheumatoid arthritis (DrugBank, 2020, and EMA, 2017). It is used as a monotherapy or in conjunction with methotrexate for individuals with moderately to severely active rheumatoid arthritis who have not reacted favorably to or are intolerant of one or more disease-modifying anti-rheumatic medications (Olumiant EPAR, EMA, 2019). Its use in conjunction with REMDS to treat hospitalized patients has been authorised by the US FDA (Jorgensen et al., 2020, and Kalil et al., 2021). Following delivery, baricitinib binds to JAK1/2 to block its activity, which also prevents the STAT signaling pathway and JAK signal transducers from being activated. Less inflammatory cytokines are generated as a result, which may postpone the onset of inflammation.Apoptosis and reduced development of cancer cells expressing are additional potential effects of Baricitinib (Saeed S, 2022, Zhang et al., 2020, and Hoang et al., 2021).

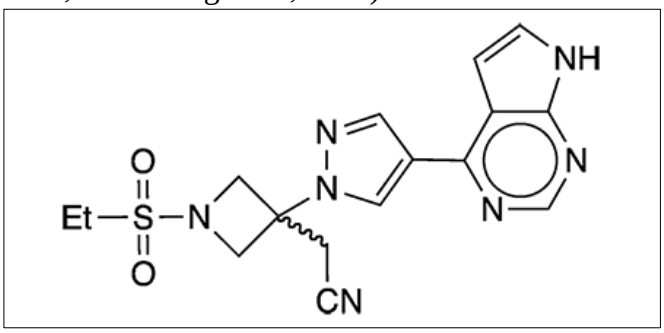

Figure 1: Structure of Baricitinib

The review of the literature indicates that the two LC techniques are not the only approaches available for figuring out the pharmacokinetics of a drug in rat plasma. The former utilized UPLC (Ezzeldin et al., 2020) while the latter used LC/MS/MS to estimate methotrexate and Baricitinib (Veeraraghavan et al., 2016). DMSO was utilized as the diluent in one UV-spectroscopic approach that was developed to determine the drug's dose form and pure form (Gandhi and Kapoor, 2019). Additionally, there aren't many HPLC methods available for determining Baricitinib. One such method uses an RPLC-Diode array detection system (Mohan, Srinivasarao and Lakshmi, 2019). Another method uses mobile phasemethanol:phosphate buffer in 45:55 ratio with a UV detector (Illendula and Prasad, 2022), and other method developed it using the QbD approach (Hamrapurkar and Mannurkar, 2021). The current procedure was created with methanol diluent and mobile phase Methanol: Water (pH-3.0 using Formic acid) in 50:50 ratio.

MATERIALS AND METHODS

*Chemicals:*The pure Baricitinib was a gift obtained from the pharmaceutical industry. Tablet dosage form Barijak which contains 4 mg of Baricitinib was bought from the local pharmacy. Solvents for HPLC used were water and methanol.

Instrument: The technique development and validation were conducted using a Shimadzu RP-HPLC.

Solvent selection: The solubility of the medication Baricitinib was examined in DMSO, DMF, and methanol, three organic solvents. During the method development process, methanol was chosen as the solvent to dissolve the medication.

Preparation of Stock solutions: 10 mg of pure Baricitinib medication was first dissolved in 2-3 ml of methanol to create a standard stock solution with a concentration of 1000 µg/ml, which was then topped off with methanol in a 10ml volumetric flask. Methanol was used to make the working standard, with a concentration of 100 µg/ml, and the following dilutions of the necessary quantities.

Calibration Curve Preparation: Methanol was used to prepare dilutions of various strengths.10 µg/ml solution was scanned in double beam UV-Visible spectrophotometer against the methanol used as blank, the λmax was determined to be 249 nm. The concentration range of 10-80 µg/ml was used to plot the calibration curve.

VALIDATION OF METHOD:

Linearity and Range: The calibration curve was used to ascertain Baricitinib's linearity. By using linear regression analysis, the correlation coefficient (r2) and the equation (y= mx+c) were found. It was also mentioned what range the medicine exhibits a linear response.

Precision: Six repetitions at the same concentration level, or 50 µg/ml, were analyzed to assess the method's repeatability. To estimate the inter-day and intra-day precision, the 50 µg/ml analysis was repeated by various analysts on different days, and by different analysts on the same day, respectively, to determine the intermediate precision. The percentage relative standard deviation is used to compute precision.

Accuracy: The method's accuracy is verified by adding 50%, 100%, and 150% of the standard drug concentration solution to the sample concentration solution, with the concentration having 20 µg/ml, 40 µg/ml, and 60 µg/ml of Baricitinib standard concentration, and 20 µg/ml respectively, and calculating the percentage recovery. Triplicate spikes were made, and the mean % recovery was computed.

Detection and Quantitation limits: The calibration standards were used to compute the quantitation and detection limits of the developed technique. In accordance with ICH rules, the detection limit was computed from the formula. Q2 R (1).

$DL = (3.3\sigma)/S.$

$QL = (10\sigma)/S.$

where σ is the response's standard deviation.

S stands for the calibration curve's slope.

Robustness: The robustness parameter was also used to validate the developed approach. A minor modification to the developed approach is evaluated. Here, a small sample of the drug's 40µg/ml solution was scanned at +/- 0.05 ml/min of the 0.50 ml/min flow rate, or 0.45 ml/min and 0.55 ml/min.

RESULTS AND DISCUSSION:

Validation of the Method:

Specificity: When a blank was introduced into the HPLC apparatus, no peaks were seen.

Linearity: Chromatograms at 249 nm were produced by injecting prepared dilutions of pure Baricitinib into the HPLC apparatus. Figure 2 shows the Gaussian-shaped peaks observed at λmax 249nm at retention time 2.205mins. The medication showed a linear response within the 10-80 µg/ml calibration curve range. The graph in Figure 3 illustrates the equation y = 79975x + 154061, for which the correlation coefficient was determined to be 0.999.

Precision: With a percentage RSD of 0.279%, the new method demonstrated good repeatability. Calculations for intra-day, and inter-day precision were done under the intermediate precision, and the results showed a percentage RSD < 2.0.

Accuracy: The developed method's accuracy was determined by calculating the recovery percentages. Table 1 displays the data from the spiked studies for accuracy. It was discovered that the mean drug recovery percentage fell between 98 and 100%.

Detection Limit (DL) and Quantitation Limit (QL): Based on the formulae outlined in ICH Q2 R (1) guidelines, the DL and QL were determined to be, respectively, 0.04677 µg/ml and 1.4174 µg/ml.

Robustness: At 0.45 ml/min and 0.55 ml/min flow rates, devised method's% RSD = 0.164% and 0.136% indicated its robustness.

Assay: The HPLC system was filled with the sample and standard solutions, and the concentration peak and peak area of the unknown material were determined. % Assay percentage was computed using formula:

$$\%Assay = \frac{Sample\ peak\ area}{Standard\ peak\ area}\ x\ \frac{Standard\ dilution\ factor}{Sample\ dilution\ factor}$$
$$x\ \frac{Average\ weight\ of\ tablets}{Label\ claim}\ x\ Potency\ of\ standard$$

% Assay = 98.55%

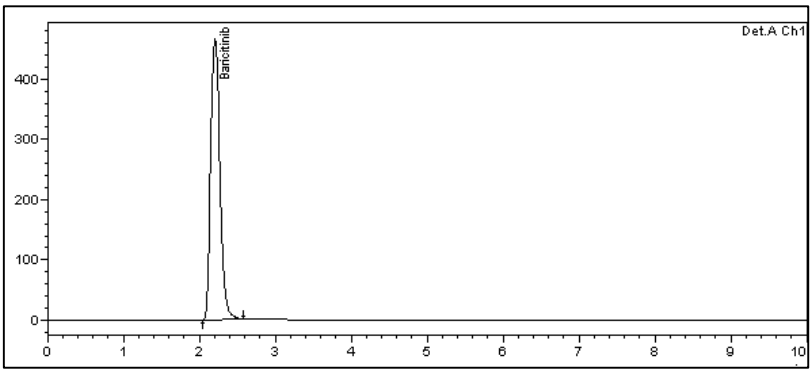

Figure 2: Chromatogram of Baricitinib.

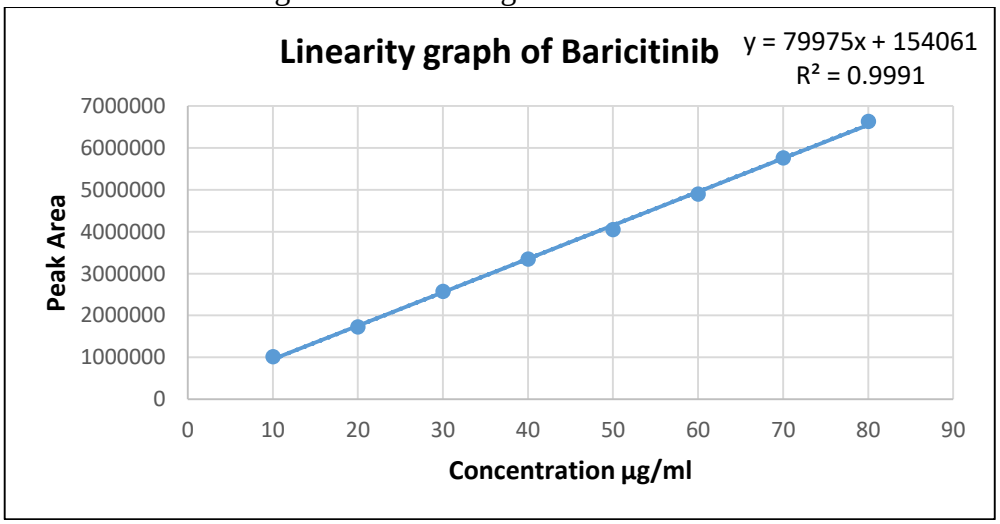

Figure 3: Linearity of Baricitinib using HPLC method developed.

Table 1: Accuracy (%Recovery) data of Baricitinib.

Spiked (%) Concentration (Std + Sample)	**Spiked Peak Area**	**Mean % Recovery**
50% 20µg/ml+20µg/ml	3287390	98.63%
100% 40µg/ml+20µg/ml	4992232	100.46%
150% 60µg/ml+20µg/ml	6706072	98.46%

CONCLUSION: It was discovered that the HPLC method for measuring and quantifying Baricitinib was straightforward, precise, linear, reliable, and fast. The concentration range of 10µg/ml to 80µg/ml was shown to exhibit linearity, with an excellent correlation value (r2) of 0.999. The results showed that DL and QL were, respectively, 0.0467µg/ml and 1.4174µg/ml. The drug recovery percentage from the spiked sample was found to be between 98 and 100%. The accuracy was also found to be within allowable limits (%RSD< 2.0). This HPLC technique is easy to use and yields precise results. The technique is useful and reasonably priced, thus it can be applied to routine quality control checks of the dosage form for Baricitinib.

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