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Original Article

STABILITY INDICATING ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF RUXOLITINIB IN BULK AND PHARMACEUTICAL DOSAGE FORM USING UPLC

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ABSTRACT

Objective: To establish a simple and selective UPLC (Ultra-high Performance Liquid Chromatography) method for the determination of Ruxolitinib in tablet and bulk dosage forms.

Methods: Chromatographic separation was achieved on a C8 column with the dimensions of (250×4.6m ID) 5 µm length; the mobile phase composition was a mixture of pH 6.2 with glacial acetic acid: Methanol: acetonitrile in the ratio of 40:30:30 was passed through the designated column with a flow rate of 1 ml per minute and the UV (Ultra Violet) detection was witnessed at 254 nm.

Results: Linearity was observed in the range 50-150 µg/ml for Ruxolitinib (r^2 =0.9998) for drugs estimated by the proposed methods was in good agreement with the label claim. The % recovery of the drug was found to be between 98 and 102%. The drug was used for determining stability studies for acid, alkali, thermal, photolytic, and peroxide degradation.

Conclusion: The method for determining Ruxolitinib was discovered to be simple, precise, accurate, and high resolution, with a shorter retention time, making it more acceptable and cost-effective for routine analysis.

Keywords: Ruxolitinib, UPLC, Linearity, Chromatographic separation high resolution, Shorter retention, Stability studies

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INTRODUCTION

Ruxolitinib is sold under the brand name jakavi or jakafi by the Incyte Corporation. This Janus-associated kinase inhibitor called Ruxolitinib, is used to treat graft vs. host disease, resistant types of polycythemia Vera, and intermediate or high-risk myelofibrosis [1, 2]. In the majority of myelofibrosis patients, Ruxolitinib therapy significantly reduces spleen size and may also help patients.

The chemical name for Ruxolitinib is 2-[4-(2, 4-[1-(2-ethoxy ethyl)-1H-1, 3-benzodiazol-2-yl] piperidin-1-yl] phenyl]-2-methylpropanoic acid, has a molecular weight of 463.622 grams per mole and the chemical formula C17H18N6. A powerful and specific JAK1 and JAK2 inhibitor, Ruxolitinib, is available. Based on the literature survey, there was no method yet published using UPLC for the determination of Ruxolitinib.

The work aimed to develop a simple and precise UPLC technique for the measurement of Ruxolitinib in a medicinal dosage form.

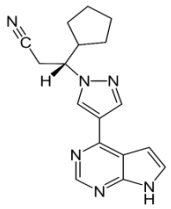

Fig. 1: Structure of ruxolitinib

MATERIALS AND METHODS

Chemicals and reagents

Samples of Ruxolitinib were obtained from Chandra labs, Hyderabad. The commercial samples of the tablet jakavi 10 mg, were provided by a local pharmacy. All the other used reagents were of analytical grade [10].

Chromatographic conditions

Chromatographic separation was achieved by using a Shimadzu 1200 UPLC system. The UV-visible spectrophotometer used was Thermo Electron Corporation. The chromatographic column utilized in these studies was Phenomenex C8 (250×4.6m ID) 5 µm. The mobile phase consists of pH 6.2 with glacial acetic acid: Methanol: ACN (acetonitrile) in the ratio 40:30:30. The flow rate selected was 1.0 ml/min with a wavelength of 254 nm. All the determinations were performed at a column oven temperature of 65 °C.

Preparation of standard solution

10 mg of Ruxolitinib was weighed and transferred into a 100 ml volumetric flask and dissolved in methanol and then make up with methanol and prepare 10 µg/ml of solution and diluted with methanol.

Assay

10 mg of Ruxolitinib was weighed in 25 ml of a volumetric flask and dissolved in 25 ml of the mobile phase and make up the volume with the mobile phase. From the above stock solution, 20 µg/ml of Ruxolitinib was prepared by diluting 0.5 ml to 10 ml with the mobile phase. Then the chromatogram was recorded.

Method development of ruxolitinib

After the development of the mobile phase and optimized chromatographic conditions, it involves the selection of a suitable wavelength.

Determination of working wavelength (λmax)

Since the drug Ruxolitinib shows maximum absorption at 254 nm as mentioned in fig. 2, it was selected for the experiment.

Optimized chromatographic conditions

Four trials were performed by changing the mobile phase compositions for better resolution. As seen in (fig. 3), the final trial was found; the Rt was observed at 3.4 min for Ruxolitinib. The peaks are sharp with less tailing; hence this method is considered final.

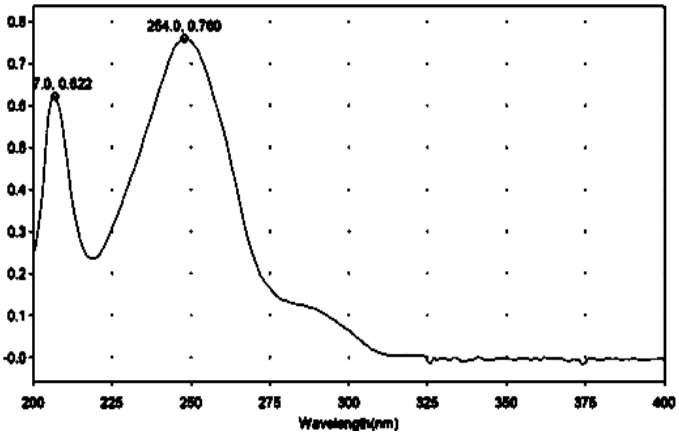

Fig. 2: Chromatogram of optimized trial

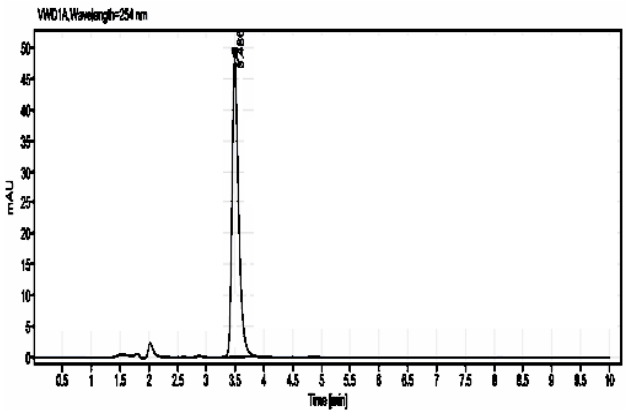

Fig. 3: Chromatogram of optimized trial

Method validation of Ruxolitinib

The developed method for Ruxolitinib was subjected to validation for the parameters like system suitability, linearity, robustness, the limit of detection (LOD), the limit of quantification (LOQ), precision, and accuracy as per the guidelines of ICH International Council for Harmonisation.

System suitability

About 10 mg of Ruxolitinib was weighed into a 10 ml volumetric flask, to this mobile phase was added sonicated, and the volume was made up to mark with the mobile phase. Further dilutions are made from standard stock solutions to get the concentration range of 100 µg/ml by pipetting 1 ml of the stock solution into a 10 ml volumetric flask.

To verify that the analytical system is working properly and can give accurate and precise results were evaluated by 100µg/ml of Ruxolitinib was injected six times and the results are obtained.

Specificity

Preparation of placebo solution

Weigh crushed Placebo powder equivalent to 100 mg of Ruxolitinib in 100 ml of volumetric flask and dissolve in 70 ml of mobile phase by 30 min of sonication and make up the volume with the mobile phase. Centrifuge the samples at 5000rpm for 10 min. From above Placebo supernatant solution was diluted from 5 ml to 50 ml with mobile phase respectively. The blank solution was also prepared and compared with the placebo.

Linearity and range

From the standard stock solution, five concentrations were prepared (50µg/ml) was prepared by taking 5 ml of stock solution taken into 100 ml of volumetric flask and diluted up to the mark with mobile phase and (80µg/ml) was prepared by taking 4 ml of stock solution was taken into 50 ml of volumetric flask and diluted with mobile phase and (100µg/ml) was prepared by taking 4 ml of stock solution was taken into 50 ml of volumetric flask and diluted with the mobile phase. The fourth concentration (120µg/ml) was prepared by taking 3 ml of stock solution was taken into 50 ml of a volumetric flask and diluting with the mobile phase. And the last concentration (150g/ml) was prepared by taking 7.5 ml of stock solution was taken into 50 ml of a volumetric flask and diluting it with the mobile phase.

Inject the samples into the chromatographic system and measure the peak area. Plot a graph of concentration (on the X-axis) versus peak area (on the Y-axis) and calculate the correlation coefficient.

Method precision

Preparation of standard solution

100 mg of Standard was weighed in 100 ml of volumetric flask and dissolve in 70 ml of mobile phase and made up the volume with mobile phase from an above stock solution 100µg/ml of Ruxolitinib was prepared by diluting 5 ml to 50 ml with mobile phase respectively.

Preparation of sample solution

10 tablets (each tablet containing 15 mg of Ruxolitinib (Brand Name: Jakavi) were weighed and taken into a mortar and crushed to a fine powder and uniformly mixed. Weigh crushed powder equivalent to 200 mg of Ruxolitinib in 200 ml of volumetric flask and dissolve in 70 ml of mobile phase by 30 min of sonication and make the volume up with mobile phase. Centrifuged sample at 5000rpm for 10 min. From the above Sample, supernatant solution prepared 100µg/ml of Ruxolitinib is prepared by diluting 5 ml to 50 ml with mobile phase, respectively.

Accuracy

For the formulation, the reference standards of the drugs (50µg/ml, 100µg/ml, and 120µg/ml) were added at the level of 50%, 100%, and 150%.

Limit of detection (LOD)

The formulaơ used for calculating LOD is mentioned below.

Limit of quantification (LOQ)

The formula used for calculating LOQ is mentioned below.

Robustness

The ability to remain unaffected by simple changes in parameters shows that the method is robust. The changes were made in terms of increasing 259 nm and decreasing wavelength 249 nm and also by making changes in the temperature.

Ruggedness

By measuring the analyst-to-analyst variance and running the Assay with two different analysts, the robustness of the technique was examined.

Degradation studies

Thermal degradation

A sample solution of Ruxolitinib (100µg/ml) was prepared and placed in the oven at 105 °C for 72 h to study dry heat degradation. Then the chromatograms were recorded.

Photolytic degradation

The photochemical stability of the drug was studied by exposing the 100µg/ml solution to UV light by keeping the beaker in a UV chamber for 24 h. For the UPLC study, the resultant solution was injected into the system and the chromatogram was recorded to assess the stability of the sample.

Acid degradation

Sample solution of Ruxolitinib (100µg/ml) prepared and transferred into a 50 ml volumetric flask and dissolve in mobile phase up to 75% then sonicate for 10 min then add 1 ml of 0.1N HCl then kept in an oven at 600c for 1 h then cool and add 1 ml 0.1N NaOH and make the volume up to 50 ml with mobile phase, then measure the chromatogram.

Alkaline degradation

Sample solution of Ruxolitinib (100µg/ml) prepared and transferred into a 50 ml volumetric flask and dissolve in mobile phase up to 75% then sonicate for 10 min then add 1 ml of 0.1N NaOH then kept in an oven at 60 °C for 1 h then cool it and add 1 ml of 0.1N HCl then make the volume up to 50 ml with mobile phase, then measure the chromatogram.

Peroxide degradation

A sample solution of Ruxolitinib (100µg/ml) and 1 ml of 20% hydrogen peroxide was mixed. For the UPLC study, 100µg/ml was injected into the system and the chromatogram was recorded to assess the stability of the sample.

RESULTS

The result of the development activity is that a suitable, easy, less time-consuming validated method has been developed for Ruxolitinib. The retention times of Ruxolitinib in the standard solution were found to be around 3.46 min and Ruxolitinib shows the percentage purity values are 100.12 %.

System suitability

System suitability parameters such as resolution, tailing factor, and no. of theoretical plates were calculated. The acceptance criteria were less than 2% relative standard deviations (RSD).

As seen in below table 1, the plate count and tailing factor results were found to be within limits and the % RSD was found to be 0.3% so the system is suitable.

Table 1: Results for system suitability of Ruxolitinib

Injection	RT (retention time)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	3.493	1593.03	32088	1.45
2	3.491	1586.84	32125	1.44
3	3.491	1591.61	32895	1.44
4	3.491	1589.38	32745	1.44
5	3.489	1592.02	31950	1.48
6	3.489	1600.39	33682	1.44
Mean	3.491	1592.21	-	-
SD		4.58	-	-
%RSD		0.3	-	-

The number of experiments are 6 and the mean of retention time RT is 3.4 and standard deviation is 4.38 and %RSD %relative standard deviation is 0.3

Specificity

It was observed that diluent or excipient peaks do not interfere with the Ruxolitinib peak. As per the results, there are no interference peaks of the mobile phase, solvent, or placebo, so we can say that the method is specific.

Linearity and range

The linearity of the method was determined by preparing five different concentrations of ruxolitinib in the concentration range of 50-150µg/ml. The calibration curves were obtained by plotting peak area versus concentration.

As the results are reported in table 2, the correlation coefficient for the linear curve obtained between concentrations vs. Area for standard preparation was found to be 0.9998. The calibration curve is shown in fig 2.

Table 2: Linearity results of ruxolitinib

Parameter	Ruxolitinib
Correlation coefficient	0.9998
Slope	16.24
Intercept	114.18

Six different concentrations were injected and the correlation coeffient (r^2) is 0.9998 and the slope s is 16.24

Precision

The method precision was determined by six injections, and the %RSD of Precision for 6 Samples determinations of Ruxolitinib was found to be within the acceptance criteria (less than 2.0%). Hence the method is precise. The method precision data is mentioned in table 3.

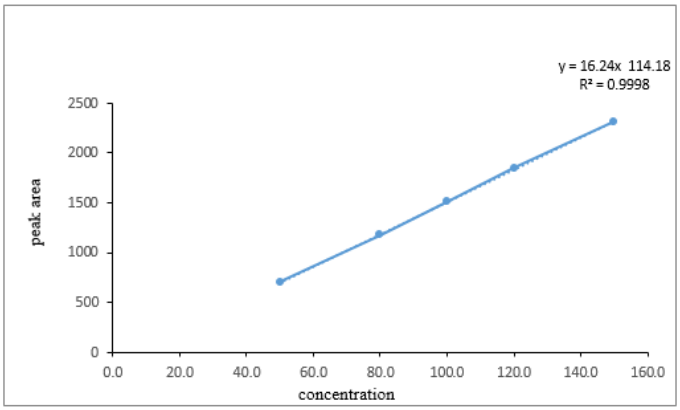

Fig. 4: Calibration curve of ruxolitinib

Table 3: Method precision data for ruxolitinib

S. No.	Area	% Assay
1	1547.86	99.20
2	1548.45	99.24
3	1554.35	99.61
4	1554.55	99.63
5	1553.78	99.58
6	1554.65	99.63
Average		99.48
SD		0.21
%RSD		0.21

The number of experiments are 6 and the average is 99.48 and Standard Deviation (SD) is 0.21 and % Relative Standard deviation %RSD is 0.21.

Accuracy

The recovery studies were carried out three times for different levels, 50%, 100%, and 150%, and the percentage recovery and percentage mean recovery were calculated for the drug. The recovery studies show the results mentioned in table 4; the percentage mean recovery of Ruxolitinib was found between 98% and 102%.

Limit of detection (LOD)

The LOD for this method was found to be 2.06 µg/ml Ruxolitinib

Limit of quantification (LOQ)

The LOQ for this method was found to be 6.26 µg/ml Ruxolitinib.

Robustness

Robustness of the method was determined. The results obtained by deliberate variation in method parameters are summarized below in table 5. As per the results, the tailing factor and theoretical plates are within the limits when the conditions are changed.

Ruggedness

It was studied by determining the analyst-to-analyst changes by performing the Assay by two different analysts. From the results mentioned in table 7. % Assay and %, RSD are under the acceptance criteria 2.0% so the method is rugged.

Degradation study results

Degradation studies were carried out as per ICH guidelines where in the thermal, photolytic, acid, basic, and peroxide, the % degradations were found to be 0.87, 0.94, 0.97, 0.91, and 0.75 % for Ruxolitinib, respectively. The results are reported in table 7, and the chromatograms of degradation studies are mentioned in fig. 5-9.

Table 4: Results for recovery of ruxolitinib

Recovery	Area	Average area	% Recovered	% Recovery
50%	1265.40	1264.72	79.43	
	1266.71			99.3
	1262.05			
100%	1589.52	1586.11	99.62	
	1584.65			99.6
	1584.18			
150%	1911.36	1917.33	120.41	
	1926.66			100.3
	1913.56			

Table 5: Results for robustness of ruxolitinib

Chromatographic changes		Tailing factor	Theoretical plates
Wavelength (nm)	249 nm	1.44	32424
	259 nm	1.47	32696
Temperature (°c)	25 °C	1.47	32474
	35 °C	1.45	32225

Table 6: Ruggedness results of ruxolitinib

Ruxolitinib	% Assay
Analyst 01	100.6%
Analyst 02	100.1 %
%RSD	0.15%

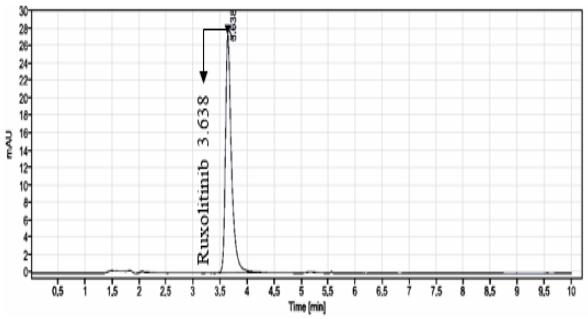

Fig. 5: Thermal degradation

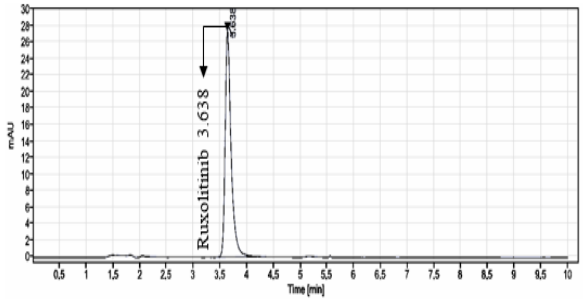

Fig. 6: Photolytic degradation

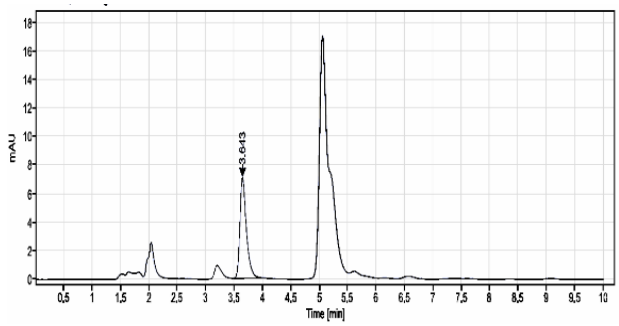

Fig. 7: Acid degradation

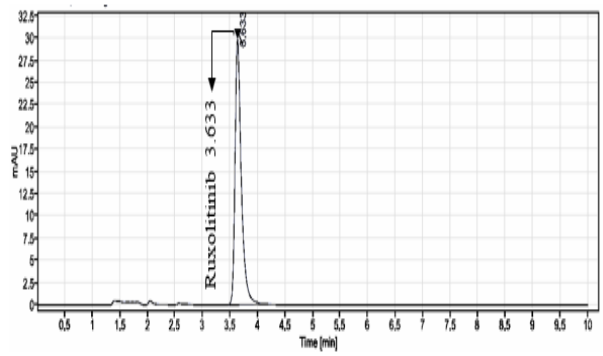

Fig. 8: Alkaline degradation

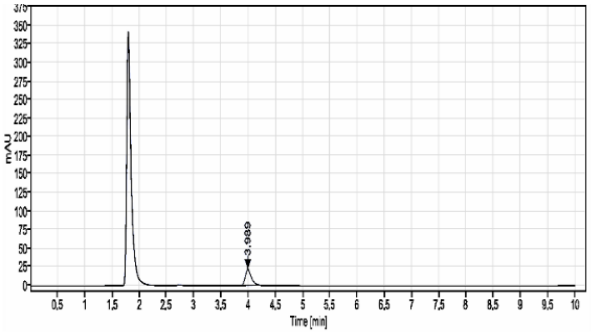

Fig. 9: Peroxide degradation

Table 7: Force degradation results of ruxolitinib

S. No.	Condition	Area	% Assay	% Degraded
1	Thermal	1623.55	99.246	0.87
2	Photolytic	1622.54	99.184	0.94
3	Acid Hydrolysis	1621.98	99.150	0.97
4	Base Hydrolysis	1622.95	99.209	0.91
5	Peroxide Hydrolysis	1625.66	99.375	0.75

DISCUSSION

Ruxolitinib is an anticancer drug that is used in the treatment of myelofibrosis and host vs graft disease [11, 12]. The suggested UPLC technique is quicker and uses less solvent than previously reported HPLC methods since its total run time was just 3.4 min, as opposed to previous HPLC methods whose run time was typically varied from 7 to 15 min [3, 4]. Additionally, the suggested UPLC approach has considerably fewer LOD (limit of detection) and LOQ (limit of quantification) values than HPLC methods previously described [5, 6], indicating that it has superior sensitivity. The values can even be modified to a lower level to improve sensitivity and resolution.

To optimize the conditions and parameters, several trials were conducted and finally, the maximum wavelength was detected at 254 nm. During the trials, different mobile phases were used like methanol: water in the ratio of 80:20 and orthophosphoric acid: acetonitrile in the ratio of 60:40, but they were not acceptable. And then glacial acetic acid: methanol: acetonitrile is used in the ratio of 40:30:30 and this shows excellent sensitivity and peak symmetry and the results are within the acceptable limits. Along with this, the column used was Phenomenex C8 (250×4.6m ID) 5 µm shows good chromatographic results. All the parameters are under the acceptance criteria mentioned in the ICH guidelines.

CONCLUSION

From the above experimental results and parameters, it was concluded that this newly developed method for the estimation of Ruxolitinib was found to be simple, precise, accurate, and high resolution and the lower retention time of this method is more acceptable and can be used for analyzing and testing in laboratories, bio-pharmaceutical, and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

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Nil

AUTHORS CONTRIBUTIONS

Dr. S. H. Rizwan carried out the analysis and interpreted the results, and Raheen Tabassum carried out the manuscript revisions.

CONFLICTS OF INTERESTS

Declared none

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