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Stability and Development of the Ruxolitinib Estimation Method Using RP-HPLC

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ABSTRACT
Ruxolitinib is a Janus Kinase 1 and 2 inhibitor drug that was designated by the Food and Drug Administration (FDA) in 2011 as a treatment for myelofibrosis with moderate to high risk. Ruxolitinib has instability to light that can cause a decrease in levels. The aim of this research is to obtain a selective, accurate and precise analytical method to determine levels and determine the stability of ruxolitinib. Determination of optimum RP-HPLC conditions from the development of analytical methods with variations in mobile phase composition, flow rate, buffer concentration and buffer pH. The new analytical method determines ruxolitinib levels by RP-HPLC using a C18 Hypersil ODS column, wavelength 310 nm, mobile phase composition acetonitrile: H2O: citrate buffer (75:20:5), injection volume 20 µL, 0.10 M citrate buffer and pH 5.8. Calibration curve between ruxolitinib concentrations in the range of 1000-1800 µg/mL, correlation coefficient 0.9997. Accuracy 99.65%, repeatability precision 0.17%, interday precision 0.36%, LOD 26.48 µg/ml, and LOQ 88.26 µg/ml. The decrease in ruxolitinib levels after exposure to acids was 82.56%, bases were 88.01%, light was 69.06%, temperature was 75.29%. So it was concluded that the method resulting from method development and validation met the validation criteria, determining ruxolitinib levels showed exposure to unstable acids, bases, temperature and light.
Keywords: ruxolitinib, method development, method validation, stability, degradation, concentration

INTRODUCTION

Ruxolitinib belongs to the class of Janus Kinase (JAK) 1 and JAK 2 inhibitor drugs which function to activate Signal Transducers and Transcription Activators (STATs) which results in the activation of the JAK STAT signal and has an impact on cell differentiation, proliferation and survival (Gerson et al., 2018). In 2011 the Food and Drug Administration (FDA) designated ruxolitinib as a treatment for moderate to high risk myelofibrosis (Mascarenhas & Hoffman, 2012).

The types of myelofibrosis included in ruxolitinib treatment include primary myelofibrosis (PMF), post-polycythemia vera myelofibrosis (post-PV MF) and essential thrombocythemia myelofibrosis (post-ET MF) (Altomare & Kessler, 2019). The active substance ruxolitinib is reported to have instability to light which is characterized by changes in the color of the active substance (Salmonson & Hemmings, 2012). Information on the stability of a medicinal product is important to know because drug instability can result in a decrease in the levels of active substances and it is feared that toxic products will arise during decomposition such as degradant products (Aashigari et al., 2019).

Drug degradation can also be influenced by the environment of the drug product and drug formulation which influence the mechanism and speed of degradation (Loftsson, 2014b). The degradation pathway has several pathways, namely, hydrolysis, oxidation, isomerization, photochemical degradation, decarboxylation, dehydration and polymerization pathways (Loftsson, 2014a). Testing the stability of an active substance or medicinal product uses forced degradation, namely using forced degradation of acids, bases, temperature and light. The results of the degradation test until the degraded product reaches approximately 20% by determining the content (Lakka, Narasimha S. Kuppan, 2020).

Until now, ruxolitinib levels have been determined using HPLC instruments. The use of HPLC has the advantage of good analysis speed, resolution and sensitivity (Suzanne Suzanne Nielsen, 2017). Previous research used tetrahydrofuran (THF) and phosphate mobile phases as buffers (Satyanarayana & Madhavi, 2012)(Charlier et al., 2019)(Biswal et al., 2019). Tetrahydrofuran has toxic properties and the use of phosphate buffers can cause abrasive effects on pump seals. Use of phosphate buffers exceeding pH 7 can shorten column life (Agrahari et al., 2013).

Based on this, to overcome the weaknesses of the previous method, the method for determining ruxolitinib levels and the HPLC method for stability testing were used. Method development and validation of HPLC methods with variations in mobile phase, flow rate, buffer concentration, buffer pH. The results of development and validation will be used if they meet the ICH validation criteria, linearity with a square correlation coefficient ≥ 0.98, precision RSD value < 2.0% and accuracy of percent return value 98-102% (GHT ICH, 2005).

RESEARCH METHODS

Material

Ruxolitinib was obtained from Dingmin Pharmaceutical of South Korea.

Preparation of citrate buffer

Sodium citrate weighed 2.94 grams and citric acid weighed 2.10 grams each dissolved in 100 ml and stirred until it reached pH 5.8.

Standard ruxolitinib preparation

100 mg of ruxolitinib standard was dissolved in methanol in a 50 ml volumetric flask. Take 5 ml of the solution and put it in a 10 ml measuring flask and add methanol to the tera mark.

Wavelength Search

Ruxolitinib solutions with three different concentrations were examined in the UV region in the range of 200-400 nm and using methanol as a blank on a UV-Vis spectrophotometer.

Sample Solution Preparation

10 mg of ruxolitinib powder was weighed and dissolved in a 10 ml volumetric flask with methanol solvent.

Chromatographic Conditions

The method used was a C18 Hypersil ODS column 250mm x 4.6 mm particle size 5µm with isocratic acetonitrile: H2O: citrate buffer pH 5.8 (75:20:5). Flow rate 1.0 ml/min, wavelength 310 nm and injection volume 20µl.

Linearity

The ruxolitinib standard solution was prepared in five concentration series from the standard ruxolitinib stock solution in the range of 1000-1800 µg/ml and then the values were processed to obtain the linear regression equation and correlation coefficient.

Accuracy

Determination of accuracy using different drug additions of 80%, 100% and 120%. An 80% concentration was prepared by dissolving 8 mg ruxolitinib in methanol in a 10 ml volumetric flask. The 100% test solution was prepared by dissolving 10 mg ruxolitinib in methanol in a 10 ml volumetric flask. The 120% test solution was prepared by dissolving 12 mg ruxolitinib in methanol in a 10 ml volumetric flask. Values are calculated to obtain an assessed recovery percentage.

Precision

Precision testing is determined using repeatability and medium precision methods. Replication was carried out six times with a concentration of 1000 µg/ml. Intermediate precision was performed on different days and values were tested against RSD values (RSD < 2.0%).

LOD and LOQ

LOD and LOQ tests are determined based on a calibration curve with several test solution concentrations, namely 1000, 1200, 1400, 1600 and 1800 µg/ml. Values are created from the calibration curve to obtain the values of σ (standard deviation of response) and S (slope of the calibration curve) which are estimated from the analyte regression line.

Robustness

The endurance test used deliberate variations with varying changes in flow rate (0.8 ml/minute and 1.2 ml/minute) and wavelength (308 and 312 nm). Retested values are percent and RSD (RSD < 2.0%).

Forced degradation of acids

The forced acid degradation test used 1000 ppm ruxolitinib solution with the addition of 1 ml of 0.1 M HCl, refluxed for 60 minutes at 70°C and reduced to room temperature. Processing degradation test data to obtain the percent reduction/loss of ruxolitinib levels by dividing the sample area after exposure by the area before exposure multiplied by 100%. LOD and LOQ testing is determined based on a calibration curve with several test solution concentrations, namely 1000, 1200, 1400, 1600 and 1800 µg/ml. Values are created from the calibration curve to obtain the values of σ (standard deviation of response) and S (slope of the calibration curve) which are estimated from the analyte regression line.

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Forced degradation of bases

The forced base degradation test used 1000 ppm ruxolitinib solution with the addition of 1 ml of 0.1 M NaOH, refluxed for 60 minutes at 70°C and reduced to room temperature. Processing degradation test data to obtain the percent reduction/loss in ruxolitinib levels by dividing the sample area after exposure by the area before exposure multiplied by 100%.

Temperature forced degradation

Temperature forced degradation test using 1000 ppm ruxolitinib solution and kept in an oven at 105°C for 60 minutes. Processing degradation test data to obtain the percent reduction/loss in ruxolitinib levels by dividing the sample area after exposure by the area before exposure multiplied by 100%.

Forced degradation of light

The forced degradation test uses a light ruxolitinib solution of 1000 ppm and is exposed to a UV chamber for 60 minutes or 200 watt hour/m2 in a photo stability chamber. Processing degradation test data to obtain the percent reduction/loss in ruxolitinib levels by dividing the sample area after exposure by the area before exposure multiplied by 100%.

RESULTS AND DISCUSSION

Wavelength Selection

Determination of the wavelength used is 2.5 ppm, 5 ppm and 10 ppm in the wavelength area of 200 – 400 nm (Table 1). At several different concentrations it shows the same wavelength, namely 310 nm.

Table 1. Determination of wavelengths with different concentrations.

Ruxolitinib Concentration (ppm)	Wave Length (nm)	Absorbance
2.5	310	0.71317
5	310	0.43316
10	310	0.25037

Chromatographic Conditions

Several variations were used to obtain the optimal method, mobile phase composition, flow rate, buffer concentration and pH. Variations in flow rate (0.8 ml/minute, 1.0 ml/minute and 1.2 ml/minute), citrate buffer concentration (0.05 M, 0.10 M and 0.15 M) and buffer pH (3.8 ; 4.8 and 5.8).

Results of the development of a method for determining ruxolitinib levels using a C18 Hypersil ODS column, flow rate 1.0 ml/minute, mobile phase composition acetonitrile: H2O: citrate buffer (75:20:5), injection volume 20 µl, wavelength 310 nm, buffer concentration 0.10 M and pH 5.8. Determination of ruxolitinib levels showed a retention time of 3,348 minutes. The tailings factor result is 1, the theoretical plate is 4483.6 and the HETP is 0.0557.

Linearity

The ruxolitinib linearity test uses five graded ruxolitinib series test solutions, namely ruxolitinib standard stock solutions of 1000, 1200, 1400, 1600 and 1800 ppm. The results of the ruxolitinib linearity test curve can be seen in (Figure 1), showing the linear regression results of $y = 25.711x + 4923.4$, R^2 value of 0.9995, r value of 0.9997.

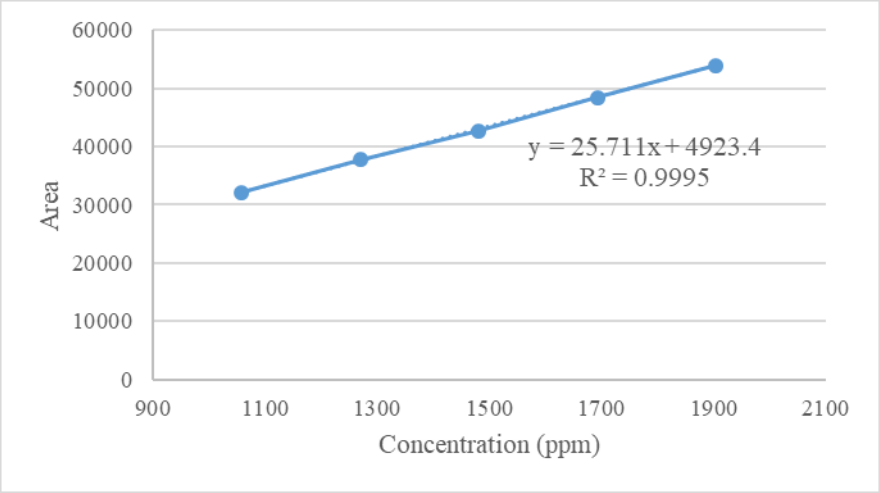

Figure 1. Graph of linearity test results

Accuracy

The accuracy test used three concentrations with three repetitions, each concentration having a percent return of 80%, a concentration of 99.46%, a percent return of 100% of 99.65% and 120%, a concentration of 99.65% with an average of 99.65% .

Precision

The repeatability precision test results obtained an RSD value of 0.17% and the medium precision test obtained an RSD value of 0.36%.

LOD and LOQ

The LOD test results were 26.48 ppm and LOQ was 88.26 ppm.

Robustness

The results of the resistance test can be seen in (Table 2) and this variation is expected to return the assessed percentage and the %RSD is not much different from before the variation was given.

Table 2. Robustness Test Results

Variation	Recovery (%)	RSD (%)
Flow rate 0.8 ml/min	99.78	0.01
Flow rate 1.0 ml/min	101.17	0.02
Flow rate 1.2 ml/min	99.01	0.01
λ 308 nm	100.82	0.01
λ 310 nm	101.17	0.02
λ 312 nm	101.18	0.01

Forced degradation of acids

The results of the forced acid degradation test resulted in a reduction in ruxolitinib levels of 82.56% and the chromatogram image can be seen in (figure 2).

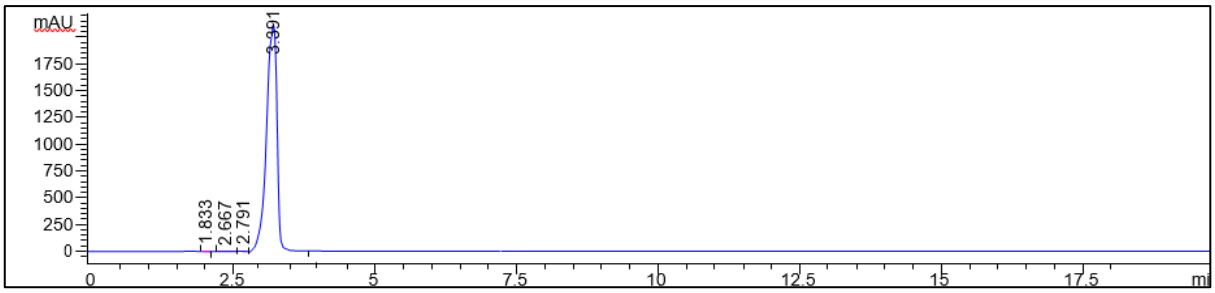

Figure 2. Chromatogram of forced acid degradation test

Forced degradation of bases

The results of the forced base degradation test resulted in a reduction in ruxolitinib levels of 88.01% and the chromatogram image can be seen in (figure 3).

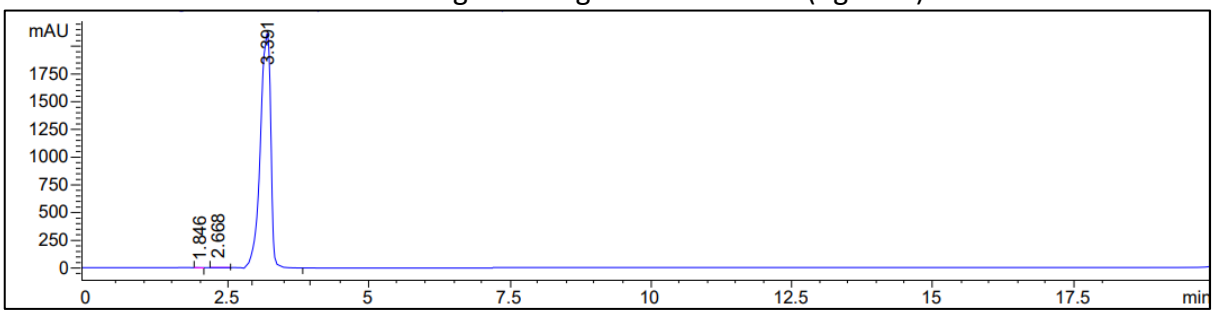

Figure 3. Chromatogram of forced base degradation test

Temperature forced degradation

The results of the forced temperature degradation test resulted in a reduction in ruxolitinib levels of 75.29% and the chromatogram image can be seen in (figure 4).

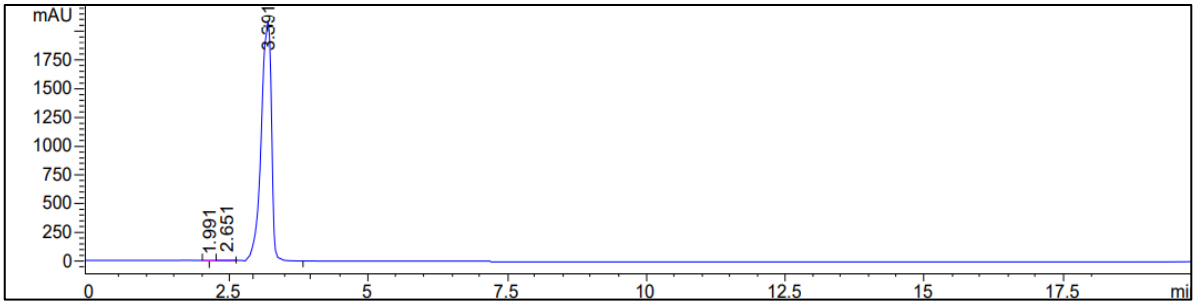

Figure 4. Forced temperature degradation test chromatogram

Forced degradation of light

The results of the force of light degradation test resulted in a reduction in ruxolitinib levels of 69.06% and the chromatogram image can be seen in (figure 5).

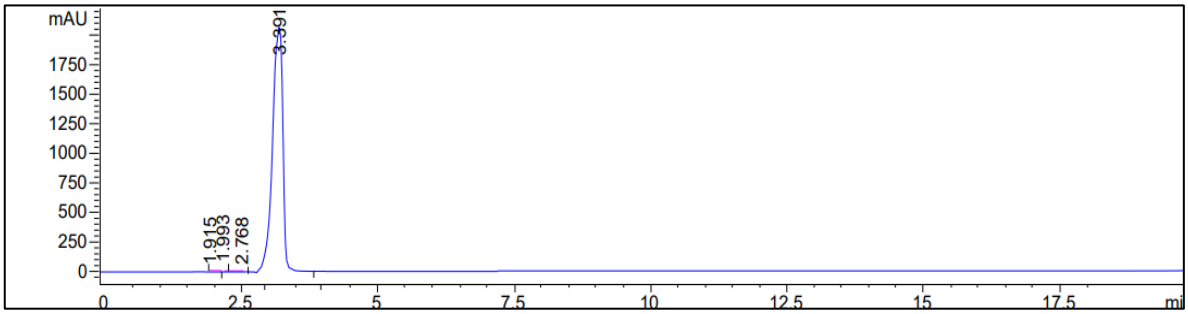

Figure 5. Forced light degradation test chromatogram

Discussion

The development of a method for determining ruxolitinib levels using an HPLC instrument begins with determining the wavelength of ruxolitinib, namely a wavelength of 310 nm. variations in chromatography test conditions to obtain a good and efficient test method. A C18 Hypersil ODS column was used, wavelength 310 nm, mobile phase consisting of acetonitrile: H2O: citrate buffer (75:20:5), injection volume 20 µl, flow rate 1ml/minute, citrate buffer concentration 0.10 M and pH 5 .8 obtained ruxolitinib retention time of 3,348 minutes. And giving a theoretical plate value of 4483.6 (>2000), HETP value of 0.0557 and a tailings factor of 1 (< 2) which shows that this value is included in the requirements(Moldoveanu & David, 2013).

The method is validated for testing to determine the validity of the method obtained. The linearity test of the method was determined using several concentrations and produced a correlation coefficient close to 1, thus indicating the method(GHT ICH, 2005). Accuracy and precision validation tests provide an average percent return value in the range of 98-102%, thus indicating an accurate method and a repeatability value expressed in % RSD of no more than 2.0% indicating an acceptable method(GH ICH, 2022). Limit of detection (LOD) and limit of quantitation (LOQ) indicate the sensitivity of the developed method. Providing a variety of methods to determine the accuracy of the method is indicated by a % RSD result of no more than 2.0% which can be interpreted as a strong method and does not provide a significant influence(GH ICH, 2022).

The results of the ruxolitinib stability test showed that light exposure caused the highest reduction in levels compared to other exposures. This decrease in levels can form new forms of decomposers according to the exposure given.

CONCLUSION

The ruxolitinib analysis method uses HPLC, namely using a C18 hypersil ODS column, at a wavelength of 310 nm, mobile phase composition acetonitrile: H2O: citrate buffer (75: 20: 5), injection volume 20 µl, buffer concentration 0.10 M and pH 5, 8. This method meets the method validation criteria according to ICH. Ruxolitinib with the influence of acid reduces levels by 82.56%, the influence of base by 88.01%, the influence of temperature by 75.29% and the influence of light by 69.06%.

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