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Original Article

Design and Development of Simple Liquid Chromatographic Technique for the Rapid Determination of Ruxolitinib (RUXO) in Bulk Drugs along with Characterization of Forced Degradation Studies

Sarangadhara Sahu[1], Susanta Kumar Panda[2,*], Sudhir Kumar Sahoo[1], Madhusmruti Khandai[1], Amaresh Prusty[3]

[1]Department of Pharmaceutical Analysis, Royal College of Pharmacy and Health Sciences, Berhampur, Odisha, INDIA.
[2]School of Pharmacy and Technology Management, SVKM's NMIMS University, Bada Bangarda, Indore, INDIA.
[3]School of Pharmaceutical Sciences-II. Siksha 'O' Anusandhan (Deemed to be University) Phulnakhara. Bhubaneswar, Odisha, INDIA.

ABSTRACT

Objectives: The motive behind this work is to develop a simple, rapid, accurate, and precise High-Performance Liquid Chromatography (HPLC) method for determining Ruxolitinib (RUXO) in bulk drugs and to study drug degradation behavior under various stress conditions as per International Council for Harmonization (ICH) guidelines**. Materials and Methods:** In this method, a Phenomenex ODS C-18 (250×4.6 mm, 5 µm) column was used by taking a mobile phase, which is methanol to acetonitrile, in the ratio of 60:40 v/v. pH is adjusted to 5.6 with acetic acid. A 1 mL/min flow rate was maintained, taking 25 µl as the injection volume. The eluted compounds were detected by a UV detector at a 254 nm wavelength at ambient temperature, with a 6 min run time. **Results:** The method shows a linear calibration curve in the concentration range of 10-70 µg/mL for the RUX with a regression coefficient of 0.999. Parameters were accessed by following the ICH (Q2R1) guideline. % Relative Standard Deviation (RSD) of precision was found to be 0.41, which is within the acceptance criteria. The Limit of Detection (LOD) value was found to be 1.5 µg/mL, and the Limit of Quantification (LOQ) is 4.5 µg/mL. The method was also utilized, developed, and validated for the estimation of drugs. Degradation tests were carried out at different conditions, like in acidic, basic, neutral, and oxidative conditions using H_2O_2. Photolytic and thermal degradation were performed at 60°C in a hot air oven. **Conclusion:** The outcomes obtained after the stress testing reveal that the RUX drug substance is stable under various stress conditions, showing its accurate, precise, rapid analysis of RUXO and hence, its suitability for routine analysis in quality control laboratories for quantitative analysis of the drug.

Keywords: Ruxolitinib, HPLC, Method Development, Validation, Force Degradation Studies, ICH Guidelines.

Correspondence:
Dr. Susanta Kumar Panda
School of Pharmacy and Technology Management , SVKM's NMIMS University, Bada Bangarda, Indore, INDIA.
Email: pandask@yahoo.com

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INTRODUCTION

Myelofibrosis in its intermediate- or high-risk state is managed by the FDA-approved selective inhibitor of Janus tyrosine kinase 1 and 2 drug, i.e., Ruxolitinib (RUXO), from the year 2011 and in the European Union in 2012.[1,2] The management includes varied conditions of myelofibrosis. The working mechanism of the drug is exhibited through Janus Kinase (JAK) and its specific subtypes, JAK1 and JAK2, where the active principle restricts signal transduction that is otherwise responsible for progression of the myelofibrotic condition.[3-5] RUXO is used in pharmacologic treatment to address repigmentation in vitiligo patients.[6] It was developed and marketed under different brand names, like Jakafi and Jakavi.[7-9] Drug having the molecular formula $C_{17}H_{18}N_6$. H_3PO_4. Its molecular weight is 404.370, and it has a pyrrolo [2,3-d] pyrimidine group in its structure. Figure 1 shows the drug's chemical structure.

The reported literature for the estimation of RUXO involves the use of LC-MS, which is not available in most of the laboratories. Similarly delayed in retention time while performing the force degradation studies, the samples are degraded.[11-14] This experiment was focused on developing a simple and accurate method for the estimation of RUXO for routine analysis and stability studies in a cost-effective manner. So, keeping pace with the current trends of pharmaceutical industries, efforts are on for the development of active principles into suitable dosage forms at a minimized cost without compromising the quality of the same.

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Analytical method development for the same active ingredient also keeps pace with the current trend. Among various methods and techniques available for determination of consistency and quality of the drug in bulk as well as in its respective dosage form, HPLC is one established, reliable analytical method. The present work designed and developed a method that can be fast, cost-effective, and reliable for the estimation of RUXO in bulk form along with performing a degradation study under various stress conditions as per ICH guidelines.

MATERIALS AND METHODS

Reagents and Chemicals

The drug was procured from Mylan Labs, Hyderabad. Methanol and acetic acid were obtained as gift samples from Nice Chemicals Pvt. Ltd., India. High-purity water was obtained by using a water purification system installed at the Royal College of Pharmacy and Health Sciences.

Instrumentation

HPLC (Shimadzu) with a UV detector and SPINCHROM data handling software was used for drug analysis. The UV-vis spectrophotometer instrument Shimadzu UV-vis 1700 was used for measuring absorbance for RUXO solutions. A pH meter and sonicator were also used during this work.

Chromatography Conditions

During this work methanol to acetonitrile in the ratio 60:40 v/v was used as the mobile phase for the ODS C-18 (250×4.6 mm, 5 µm) column. Before the start of experimental work, the column was conditioned by taking the selected mobile phase. pH is adjusted to 5.6 with acetic acid. The injection volume was set to 25 µL with a 1 mL/min flow rate.

Selection of Wavelength

The drug shows absorbance and good resolution at a 254 nm wavelength.

Preparation of Mobile Phase

Preparation of the mobile phase holds a crucial role in the method validation process. In the present study, a mixture of methanol and acetonitrile was selected as the mobile phase. 600 mL of methanol along with 400 mL of acetonitrile was mixed well and then degassed, which was filtered through a 0.45 µm filter using vacuum filtration.

Standard Stock Solutions

A standard stock solution was prepared by accurately measuring 10 mg of the drug and dissolving it in 10 mL of the selected mobile phase to get a concentration of 1 mg/mL (1000 µg/mL) solution and stored under refrigeration.

Working Solutions

2.5 mL of stock solution was taken in a 25 mL volumetric flask and diluted to get a concentration of 100 µg/mL solution. From this, 1 mL, 2 mL, 3 mL, 4 mL, 5 mL, 6 mL, and 7 mL were taken to make 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, 50 µg/mL, 60 µg/mL, and 70 µg/mL solutions, respectively, by diluting with methanol and acetonitrile solutions of ratio 60:40, respectively, and then injected into the HPLC in a 1 mL/min flow rate.

Method validation of RUXO

The present research aims to develop and validate the determination of RUXO as per ICH guidelines, analyzing different parameters like linearity, precision, robustness, LOD, and LOQ.

Linearity

Working solutions are injected in series from lower concentrations to higher concentrations to check linearity of response, and peak areas are recorded. Different concentrations ranging from 10 to 70 µg/mL was prepared by diluting the stock solution, and a graph was plotted.

Accuracy

Accuracy was found out by taking different sample solutions at various levels such as 80%, 100% and 120% to determine the % recovery of RUXO by calculating mean, SD and % RSD by standard addition method.

Precision

To express and calculate repeatability and intermediate precision study, eight working sample solutions of 40 µg/mL are injected. The % RSD and standard deviation were calculated for RUXO for both intra-day and inter-day precision.

LOD and LOQ

The calibration curve method was used in the determination of these parameters to check the sensitivity of the method. Signal-to-Noise ratios (S/N) of 3:1 and 10:1 are used to determine LOD and LOQ, respectively, by formula.[15-17]

$$LOD = 3X \text{ standard deviation of y- intercept / slope of calibration curve}$$

$$LOQ = 10X \text{ standard deviation of y- intercept / slope of calibration curve}$$

Ruggedness

Ruggedness was determined based on an internalist study, where two different analysts were employed to perform the experimentation and interpretation of data thereof. The results are represented in Table 3.

Robustness

The robustness was assessed by introducing the RUXO standard configuration into the HPLC while varying the flow rate, altering the pH, and modifying the composition of the organic solvent from standard chromatographic conditions.

Degradation Studies

It is necessary to demonstrate the specificity of stability-indicating methods, which also offers insight into the degradation pathways and products of the drug substance, aiding in the elucidation of the structures of the degradation products. The authors adhered to a specified procedure for conducting the degradation study. The sample underwent these conditions; the principal peak was analyzed for peak purity, confirming that the procedure effectively isolated the pure active component from the breakdown products.

Degradation in Natural Condition

About 10 mg of pure drug was accurately weighed and taken in a 10 mL volumetric flask and dissolved in a minimum volume of methanol. Then the volume was made up to the mark with water and kept at 70°C. At different time intervals, solutions were prepared and injected into the HPLC system.

Acid Degradation

10 mg of pure drug taken in a 10 mL volumetric flask and dissolved in a minimum volume of methanol and sonicated for 10 min. Then the volume was made up to the mark with 1 mL of 0.1 N HCl and kept at 70°C. Then this solution was then cooled, neutralized by 1 mL of 0.1N NaOH, and injected under optimized chromatographic conditions to study the nature of the chromatogram.

Table 1: Optimized Chromatographic Conditions of RUXO phosphate.

Parameters	Conditions
Column	ODS-II C_{18} (250×4.6 mm, 5µ)
Mobile Phase	Methanol: Acetonitrile (60:40) with Acetic Acid
Flow rate (mL/min)	1 mL/min
Run time (minutes)	6 min
Column temperature (°C)	Ambient
Volume of injection loop (mL)	25 mL
Detection wavelength (nm)	254 nm
Drug R_t (min)	3.087
LOD	1.5µg/mL
LOQ	4.5µg/mL

Basic Degradation

10 mg of pure drugs were accurately weighed and taken in a 10 mL volumetric flask and dissolved in a minimum volume of methanol and sonicated for 10 min. Then the volume was made up to the mark with 0.1N NaOH and kept at 70°C. Then this solution was then cooled, neutralized by 1 mL of 0.1N HCl, and injected under optimized chromatographic conditions to study the nature of the chromatogram.

Oxidative Degradation

About 10 mg of pure drugs were accurately weighed and taken in a 10 mL volumetric flask and dissolved in a minimum volume of methanol. Then the volume was made up to the mark with 6% w/v H_2O_2 and kept at 70°C. Then this solution was then cooled and injected under optimized chromatographic conditions to study the nature of the chromatogram.

Thermal Degradation

Three different clean Petri dishes containing approximately 10 mg of pure drugs were heated to 700°C using dry heat. Drug solutions were made, and 40 µg/mL of the sample solutions were added to the HPLC apparatus.

RESULTS

Method Development

As we know, if drugs are not given in the correct quantity, they may produce adverse effects in the body. So, developing a simple, rapid, cost-effective analytical tool for its estimation has always been a thrust area of research that may guarantee that the drug exists in the exact concentration in the sample. In this line, this research work reports a new, rapid, simple, and low-cost HPLC analytical tool with a UV detection channel for estimating RUXO in bulk samples and also estimates the effect of stressed conditions on the degradation property of RUXO. The method was successfully applied for the estimation of the drug, and the result of optimized chromatographic conditions is shown in

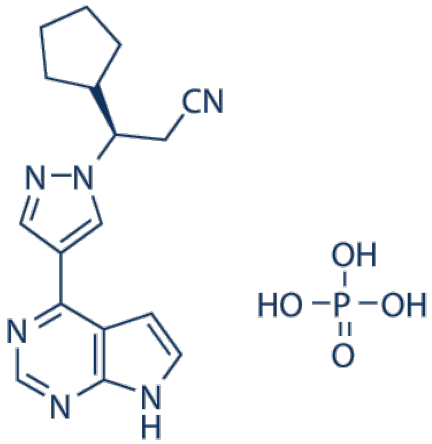


Figure 1: Chemical structure of Ruxolitinib.[10]

Table 2: Accuracy Data of UV-vis Spectrophotometric Method for RUXO.

No. of preparation	Formulation	Pure Drug	% Recovery	Statistical Parameter
S1:80%	10	8	99.98	Mean=100.1
S1:80%	10	8	100.5	SD=0.344
S1:80%	10	8	99.85	% RSD=0.344
S1:100%	10	10	100.8	Mean=99.91
S1:100%	10	10	99.65	SD=0.916
S1:100%	10	10	98.99	% RSD=0.918
S1:120%	10	12	99.97	Mean=100.13
S1:120%	10	12	100.01	SD=0.249
S1:120%	10	12	100.42	% RSD=0.249

Table 3: Precision Result of RUXO.

Precision Parameters	Statistical Parameter		
	Mean	SD	%RSD
Precision data of Repeatability	39.92	0.16	0.41
Intra-day Precision	40.00	0.04	0.12
Inter-day Precision	39.97	0.15	0.39

The numbers of readings are 6.

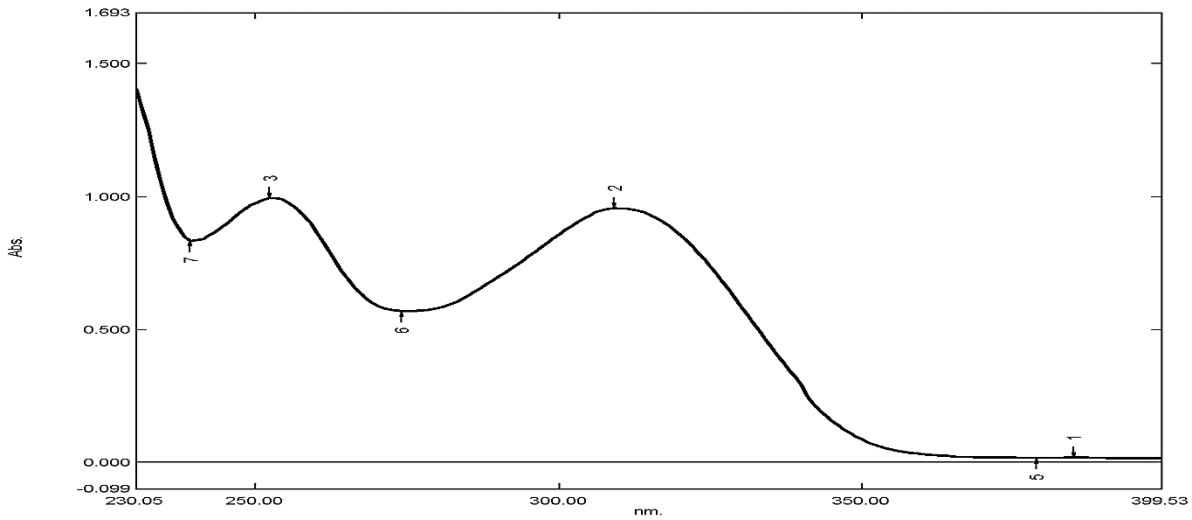


Figure 2: UV spectrum of RUXO.

Table 1. ODS-II C18 (250×4.6 mm, 5µ) was chosen as appropriate for this method. The eluted RUXO shows good peak shape and a retention time of 3.087 min by using a mixture of methanol to acetonitrile in 60:40 ratios with acetic acid. Flow rate was maintained at 1 mL/min. The drug shows its peak at 254 nm, as shown in Figure 2 and the optimized chromatogram of RUXO shown in Figure 3.

Method Validation

The results of linearity occur within the range of 10-70 µg/mL that shows the straight-line equation of y=29163x-10749 with the correlation coefficient found to be 0.9996 for RUXO. From the regression equation, LOD and LOQ were found to be 1.5 µg/mL and 4.5 µg/mL, respectively, as mentioned in Table 1. Different validation parameters such as accuracy, precision, robustness, ruggedness, LOQ, LOD were studied according to ICH guidelines and the corresponding data are given in Table 2.

In the precision study of repeatability, the % amount found was calculated. The outcome of the results of precision was shown in Table 3, and the linearity of the chromatogram RUXO is shown in Figure 4. In the robustness study, % RSD for selected chromatographic parameters like changes in flow rate (±0.2 mL/min), mobile phase composition (±5%), and room temperature (±5°C) are found to be less than 2.0, as shown in Table 4. Ruggedness was done by different analysts, and the result is shown in Table 5. The degradation study was conducted to check the effect of acid, base, oxidation, thermal, photolytic, and water on

RUXO. The results are shown in Table 6 and the chromatogram shown in Figure 5.

DISCUSSION

From the calibration plot of response vs. concentration, a straight line follows that can be used to evaluate a procedure's linearity. The eluted RUXO shows good peak shape and a retention time of 3.087 min by using a mixture of methanol to acetonitrile in 60:40 ratios with acetic acid. Flow rate was maintained at 1 mL/min. The drug shows its peak at 254 nm, as shown in Figure 2. In the precision study of repeatability, the % amount found was calculated. The %RSD of precision was found to be 0.41, which is within the acceptance criteria (less than 2.0%). Hence, the method is precise, as shown from results table. In the photolytic and thermal degradation study, the drug has not shown any extra peak, confirming its stable nature. From the Figure, it has been confirmed that, whatever the degradation patterns, RUXO was eluted at its specific peak at its own retention time, and there

Table 4: Robustness Result of RUXO.

Change in Parameters	%RSD
Flow (1.1 mL/min)	0.15
Flow (0.8 mL/min)	0.21
Temp. (28°C)	0.17
Temp. (32°C)	0.89
More Organic	0.12
Less Organic	0.15

The numbers of readings are 6.

Table 5: Ruggedness Result of RUXO.

Change in Parameters	%RSD
Analyst - 1	0.26
Analyst - 2	0.42

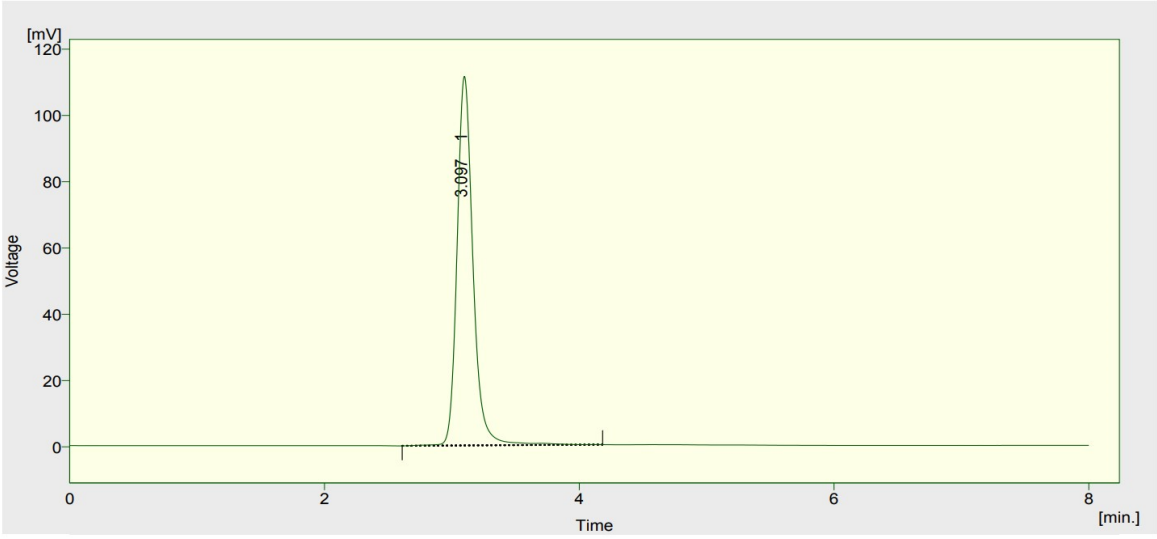


Figure 3: Optimized chromatogram of RUXO.

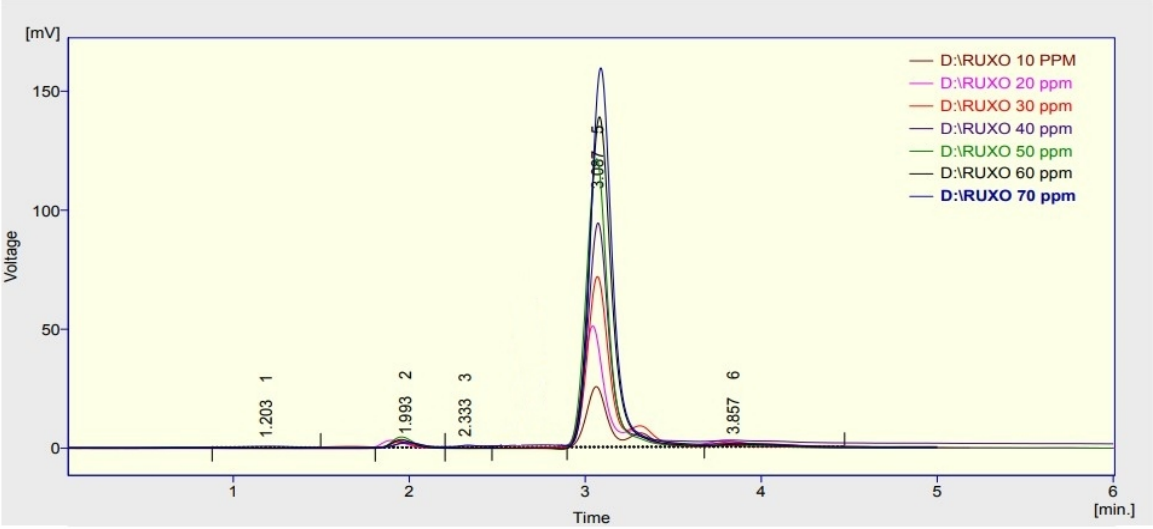
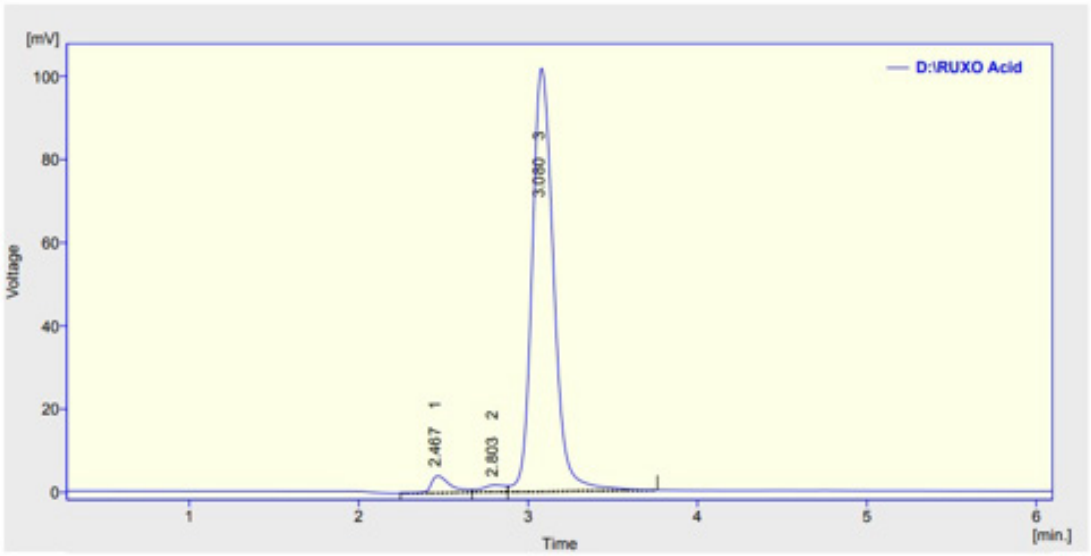
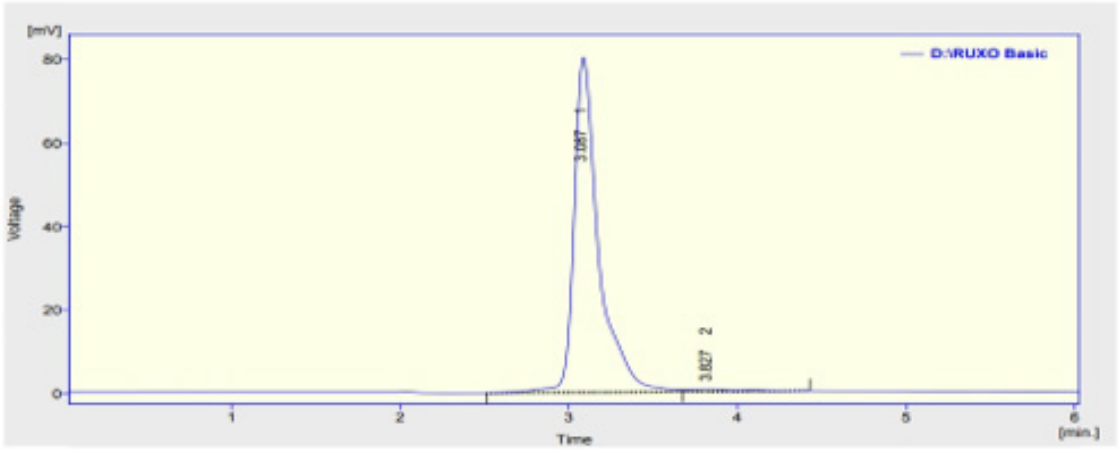


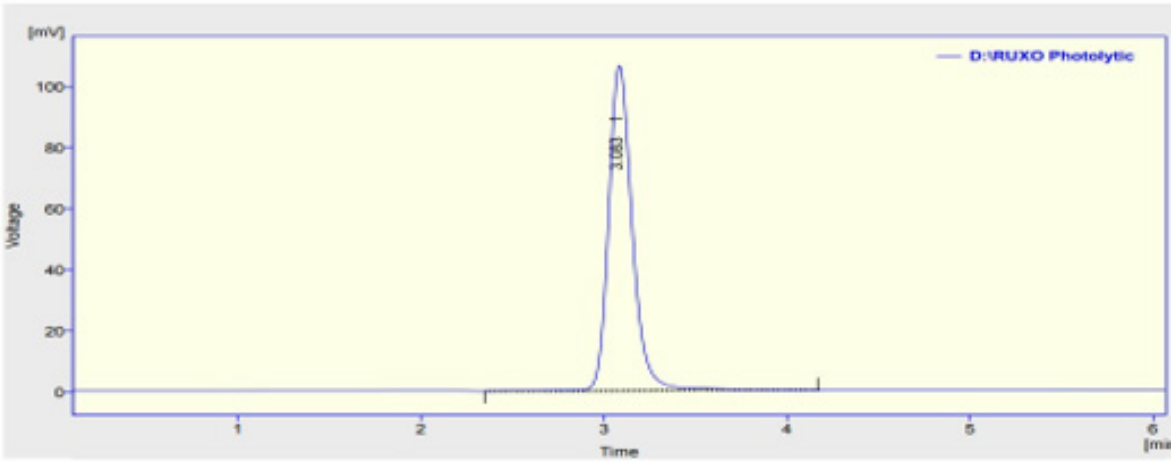
Figure 4: Linearity Chromatogram of RUXO.


[mV]
D:\RUXO Acid
Voltage
2.467 1
2.803 2
3.080 3
Time
[min.]

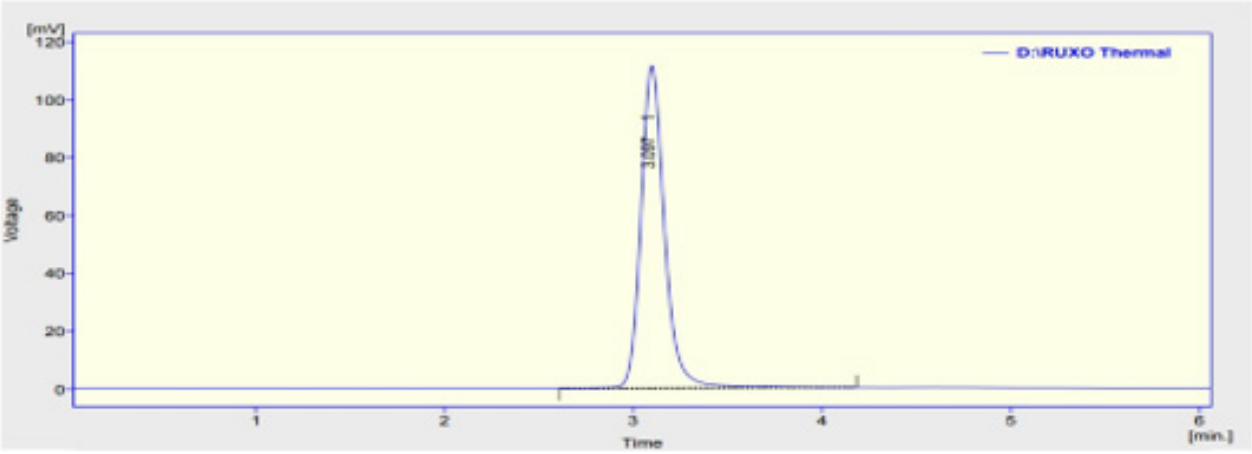
a


[mV]
D:\RUXO Basic
Voltage
3.057 1
3.827 2
Time
[min.]

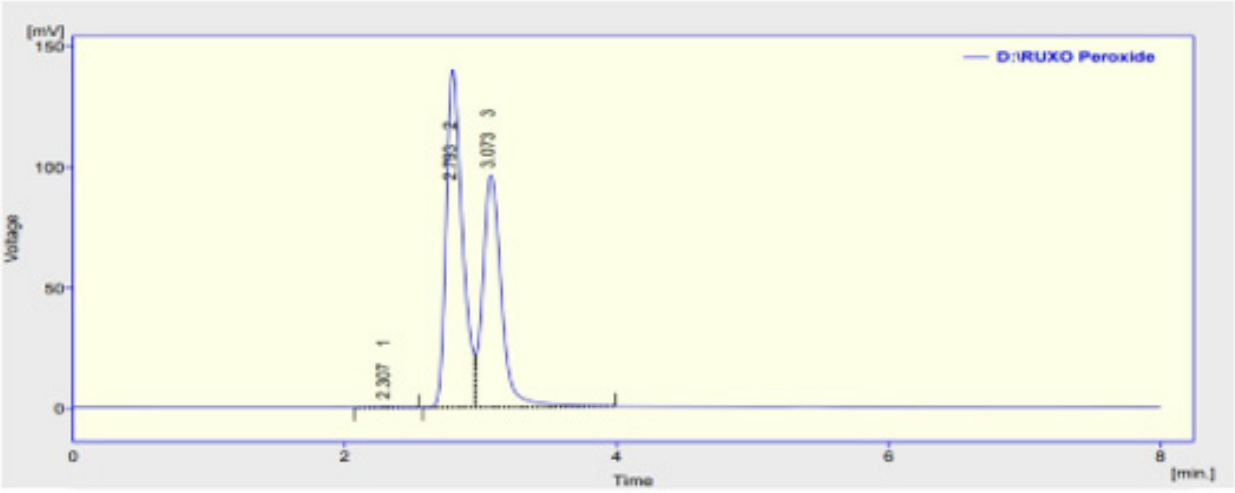
b


[mV]
D:\RUXO Photolytic
Voltage
3.053 1
Time

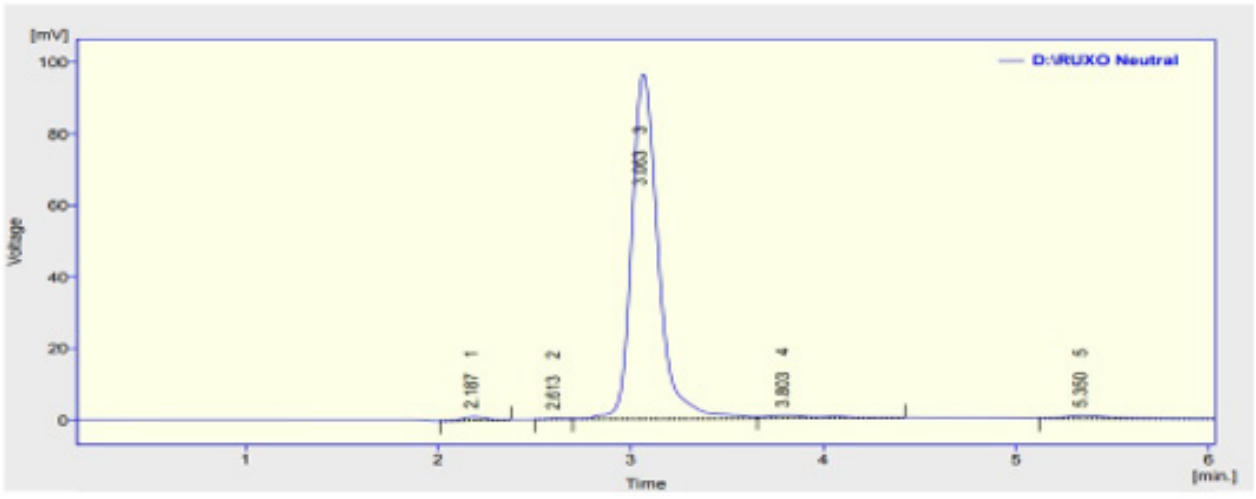
c



d



e



f

Figure 5: Chromatogram of Degradation study at different Conditions. (a) Acid degradation (b) Basic degradation (c) Photolytic degradation (d) Thermal degradation (e) Peroxide degradation (f) Neutral Degradation.

Table 6: Results of Forced Degradation study of RUXO phosphate.

Stressed Parameters	% Assay of RUXO	Findings
Acid degradation	99.09	There was no significant degradation found.
Alkali degradation	98.57	There was no significant degradation found.
Photolytic degradation	99.48	There was no significant degradation found.
Thermal degradation	97.73	There was no significant degradation found.
Peroxide degradation	81.90	Degradation peak of 17.66% was formed.
Neutral Degradation	99.90	There was no significant degradation found.

are no new extra sharp peaks observed in different stressed conditions, which confirms its stable nature.

CONCLUSION

This research work reports on the development of a simple, accurate, precise, and rapid HPLC method for the estimation of Ruxolitinib (RUXO) in bulk. While performing the forced degradation studies, it is found that RUXO remains stable in most of the stress conditions. However, while formulating, the pH should be kept near to neutral to optimize the stability of the product. This method is "rapid" as it reduces the whole analysis time, which is the smallest amount of time needed. Since there has been reduced deterioration in stressful environments and good separation of RUXO from the other deteriorated peaks, the current technique is "stability indicating." The procedure is "validated," since all parameter results are within the limit specified by the ICH Q2B. From the system development and confirmation data, it's set up that the system is specific, accurate, precise, rugged, and robust for the estimation of RUXO. Hence, it can be used for the routine analysis of RUXO in different labs.

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ABBREVIATIONS

RUXO: Ruxolitinib; **ICH:** International council for harmonization; **HPTLC**: High performance Liquid chromatography; **LC-MS:** Liquid chromatography-mass spectrometry; **RSD:** Relative standard deviation; **LOD:** Limit of detection; **LOQ:** Limit of quantitation.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

This research work reports on the development of a simple, accurate, precise, and rapid HPLC method for the estimation of Ruxolitinib (RUXO) in bulk. While performing the forced degradation studies, it is found that RUXO remains stable in most of the stress conditions. The procedure is "validated," since all parameter results are within the limit specified by the ICH Q2B. From the system development and confirmation data, it's set up that the system is specific, accurate, precise, rugged, and robust for the estimation of RUXO. Hence, it can be used for the routine analysis of RUXO in different labs.

REFERENCES

1. Masarova L, Mascarenhas J, Rampal R, Hu W, Livingston RA, Pemmaraju N. Ten years of experience with ruxolitinib since approval for polycythemia vera: A review of clinical efficacy and safety. Cancer. 2025;131(1):e35661. doi: 10.1002/cncr.35661
2. Rodrigues MA, Torres T. JAK/STAT inhibitors for the treatment of atopic dermatitis. J Dermatolog Treat. 2020;31: 33-40. https://doi. org/ 10. 1080/ 09546 634. 2019. 15775 49.
3. Passamonti F, Palandri F, Saydam G, et al. Ruxolitinib versus best available therapy in inadequately controlled polycythaemia vera without splenomegaly (RESPONSE-2): 5-year follow up of a randomised, phase 3b study. Lancet Haematol. 2022;9(7):e480-e492. doi: 10.1016/s2352-3026(22)00102-8
4. Koschmieder S, Isfort S, Teichmann LL, et al. Firstline treatment with ruxolitinib versus best available therapy in patients with polycythemia vera. Paper presented at: 65th American Society of Hematology (ASH) Annual Meeting and Exposition; December 9-12, 2023; San Diego, CA
5. Cervantes, F., and Martinez-Trillos, A. (2013). Myelofibrosis: an update on current pharmacotherapy and future directions. Expert opinion on pharmacother. 2003;14(7): 873-884. https://doi.org/10.1517/14656566.2013.783019
6. FDA approves topical treatment. U.S. Food and Drug Administration (FDA) (Press release). 19 July 2022. Archived from the original on 2022. Retrieved 20 December 2022.
7. Solimani F, Meier K, Ghoreschi K. Emerging topical and systemic JAK inhibitors in dermatology. Front Immunol. 2019;10:2847. https:// doi. org/ 10. 3389/ fimmu. 2019. 02847.
8. JAKAFI (ruxolitinib). Full Prescribing Information. Incyte Corporation, Wilmington; 2023.
9. Ruxolitinib (Opzelura) for atopic dermatitis. Med Lett Drugs Ther. 2022; 64(1642):12-13. PMID: 35134043.
10. Haq M, Adnan G. Ruxolitinib. [Updated 2023 Jun 5]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025.
11. Charlier, B., Marino, L., Dal Piaz, F., Pingeon, M., Coglianese, A., Izzo, B., et al. Development and Validation of a Reverse-Phase High-Performance Liquid Chromatography with Fluorescence Detection (RP-HPLC-FL) Method to Quantify Ruxolitinib in Plasma Samples. Analytical Letters, 2019;52(8):1328-39. DOI:10.1080/ 00032719.2018.153728
12. Tabassum R, Rizwan SH. Stability indicating analytical method development and validation for the estimation of Ruxolitinib in bulk and pharmaceutical dosage form using uplc, ijpps, 2023;15(2) DOI: 10.22159/ijpps.2023v15i2.46839

13. Biswal S, Mondal S, Mondal P. A New Stability Indicating High Performance Liquid Chromatography Method for the Estimation of Ruxolitinib in Bulk and Tablet Dosage Form, Pharm Methods, 2019;10(2):53-7, DOI: 10.5530/phm.2019.2.10.
14. Samal L, Prusty A. Development and Validation of UV-Visible Spectrophotometric Method for Determination of Duloxetine. Int J Pharm Pharm Sci, 2019;11(3):27-31
15. Behera B, Mishra US, Sahoo SK, Development and Validation of Stability Indicating RP-HPLC Method for The Analysis of Vilazodone in Bulk form and Marketed Pharmaceutical Dosage Form, Research Journal of Pharmacy and Technology 2023;16(9):4319-4. doi: 10.52711/0974-360X.2023.00707
16. Bisoyi SK, Pradhan D, Sahoo SK. Quantitative Estimation of Eltrombopag in Bulk and Tablet Dosage Form by UV- Spectroscopy. Research Journal of Pharmacy and Technology. 2025;18(5):2101-6. doi: 10.52711/0974-360X.2025.00301.
17. Sahoo SK., Choudhury PK., Murthy PN., Mishra US, Bisoyi SK, Kumar L. Systematic approach to develop and validate High Performance Liquid Chromatographic method for efavirenz and its degradants. Journal of Applied Pharmaceutical Research. 2024;12(3):119-128.

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