RJC

RASĀYAN *J. Chem.*
Vol. 16 | No. 3 |1163-1173| July - September | 2023
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF BARICITINIB AND ITS RELATED DRUG SUBSTANCES

Kiran Kumar Gannamani and Sudhakar Chintakula✉
Department of Chemistry, School of Science, GITAM (Deemed to be University), Visakhapatnam-530045, Andhra Pradesh, India
✉Corresponding author: schintak@gitam.edu

ABSTRACT

The current study aims to develop a method for determining Baricitinib along with its related compounds, as well as to confirm its stability under various circumstances and forced degradation methods using reverse-phase HPLC. The separation was performed using an inert-sustain C8 column that is with a 4.6 mm internal diameter, 250 mm long, which is loaded with Octyl silane chemically bound to porous silica particles of 5 µm diameter, with a flow rate of 1 ml per minute, UV detection of 225 nm, and a column oven temperature of 45 °C. The total time required to acquire data was fifty minutes. The test method has been validated and shown to be accurate in terms of specificity, precision (system, method, and intermediate precision), linearity, the limit of detection (LoD) and limit of quantitation (LoQ), accuracy, range, stability of mobile phase, standard and sample solutions, and system suitability. For the purpose of identifying related compounds in Baricitinib drug material, the devised test method is appropriate and can be used routinely.

Keywords: Degradation, Method Development, Impurities, RP-HPLC, Baricitinib, Validation, Stability.
RASĀYAN *J. Chem.*, Vol. 16, No. 3, 2023

INTRODUCTION

Baricitinib is an inhibitor of Janus kinase (JAK) that inhibits both JAK1 and JAK2 subtypes[1]. It is typically used to treat rheumatoid arthritis;[2] however, its recently discovered anti-viral properties and efficacy in treating mild to severe COVID patients have garnered more attention[3,4,5]. This substance is known by its chemical name, "2-[1-Ethylsulfonyl-3-[4-[7H-pyrrolo [2,3-d] pyrimidin-4-yl] pyrazol-1-yl] azetidin-3-yl] acetonitrile" according to IUPAC nomenclature, with molecular formula $C_{16}H_{17}N_7O_2S$ and molecular mass 371.4 g/mol[6]. Baricitinib's related substances are Baricitinib carboxamide, Baricitinib methyl ester, Pyrrolopyrimidine Baricitinib, Azetidinyl Baricitinib, and Baricitinib methyl pivalate (Fig.-1 to 6), which are used as important ingredients in the preparation of various pharmaceuticals.

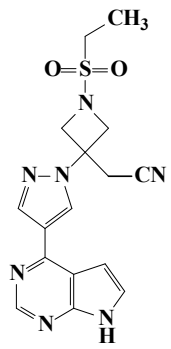


Fig.-1: Chemical Structure of Baricitinib

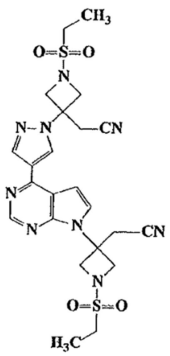


Fig.-2: Chemical Structure of Azetidinyl Baricitinib

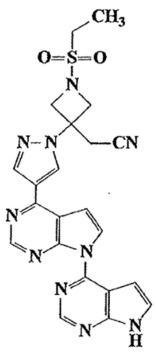


Fig.-3: Chemical structure of Pyrrolopyrimidine Baricitinib

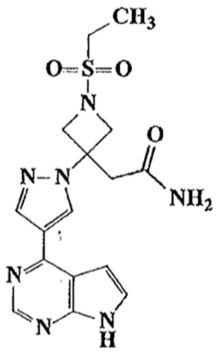


Fig.-4: Chemical Structure of Baricitinib Carboxamide

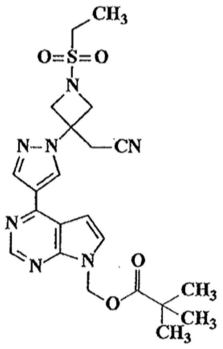


Fig.-5: Chemical Structure of Baricitinib Methyl Pivalate

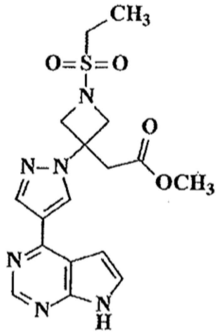


Fig.-6: Chemical Structure of Baricitinib Methyl Ester

Few researchers developed estimation methods for Baricitinib using LC-MS, RP-HPLC, spectroscopy, and Quality by Design (QbD)[7-10]. However, there are limited studies validating the methods devised for determining Baricitinib and its related compounds. Consequently, the objective of the present study was to devise a method for determining Baricitinib and its related compounds and to validate its stability under various conditions and forced degradation methods using reverse-phase HPLC.

EXPERIMENTAL

Instruments

A High-Performance Liquid Chromatography (HPLC) system with a Waters 2695 separations module and 2489 UV/Vis detector (Waters Alliance or equivalent), a data handling system (Empower or equivalent), and a Ghost-Guard LC column for gradient elution in reverse phase HPLC, where a mobile phase cleaning column with a 4.6 mm internal diameter and length of 30 mm was used.

Preparation of Solutions

Standard and Sample

The standard Baricitinib was procured from Simson Pharma, and samples were prepared in-house using chemical synthesis.

Buffer

1000 ml of water was mixed with 1.36 g of potassium di-hydrogen ortho-phosphate, and KOH solution was used to get the pH to 6.0 ± 0.05.

Mobile Phase

Acetonitrile was used for mobile phase B, whereas Buffer and Acetonitrile were combined in a 95:5 v/v degassed mixture to make the mobile phase.

Diluent

A degassed mixture of water and Acetonitrile was prepared at 50:50 v/v.

System Suitability Solution

About 5 mg of Baricitinib reference sample [containing Baricitinib methyl ester, Azetidinyl Baricitinib, and Baricitinib methyl pivalate] dissolved in 10 ml of diluent.

Impurity Stock Solution

2.0 mg each of Baricitinib methyl ester, Azetidinyl Baricitinib, and Baricitinib methyl pivalate was added to 2 ml of Acetonitrile was added and dissolved by sonication.

Standard Stock Solution

Approximately 50 mg of Baricitinib WRS was mixed with 70 ml (equivalent to 1.4 mg/ml) of diluent and dissolved by sonication. From this solution, 5 mL was added to the diluent to make up the final volume of 100 mL to obtain Baricitinib standard solution (70 μg/ml).

Standard Solution
Diluent was added to 3 ml of standard stock solution to make up to 100 ml.

Sensitivity Solution
standard stock solution (1ml) was mixed with 100 ml of diluent.

Sample Solution
50 mg of the sample was dissolved in 70 ml of diluent by sonication.

Method Development
Numerous attempts have been made to detect and validate Baricitinib, utilizing various column, buffer, and mobile phase concentrations.

Validation[8]
Specificity
Using Waters Empower software, the purity of the peak was determined. To validate the retention time, solutions of each known related compound were prepared and injected one at a time. In accordance with the protocol, diluent solutions were prepared and injected into HPLC alongside the Baricitinib drug (control) and test substances that had been contaminated with known related compounds at a specific concentration (spiked sample).

System Suitability
Individual injections of diluent, sensitivity, system suitability, and standard solutions into the chromatograph were made six times, and the results were recorded. The system's suitability and stability were evaluated daily using the recorded relative mean and standard deviation.

LoD and LoQ
The signal-to-noise ratio was utilized for calculating LoD and LoQ. On the basis of the analyte's peak response, the LoD and LoQ values of Baricitinib and its associated compounds (known concentration) were determined using S/N ratios of 3:1 and 10:1, respectively. The accuracy of LoD and LoQ was confirmed by preparing solutions with Baricitinib and its related compounds at expected concentrations and each solution was injected six times into HPLC.

Precision
Using the test method, a standard solution was prepared and injected six times into the HPLC to determine the system's precision. Six sample solutions from a single batch of baricitinib loaded with known related compounds were used to evaluate the accuracy of the procedure. In addition, different analysts prepared and analyzed identical set solutions from the same sample (same batch used in method precision) on various days using a distinct system and columns to calculate the intermediate precision (inter and intraday precision).

Linearity
Baricitinib and its related compounds were utilized to produce solutions with concentrations ranging from 5% to 120% of the specified level. Each solution was then injected six times into the HPLC. Using Response (Area) versus Concentration (Amount), a calibration curve was constructed. Using the graph, the y-intercept and correlation coefficient (r2) were determined.

Accuracy
Before each solution was injected into the HPLC, triplicates of baricitinib drug substance were spiked with known related compounds at LOQ, 50%, 100%, and 120% of specification levels. Using the peak area values derived from the calibration curve equation, the recovered concentration values and recovery percentage were calculated.

Solution Stability
The stability of the sample, standard, and mobile phase was determined by injecting them with known related substances at a predetermined concentration and analyzing sing them at various time intervals. In addition to analyzing the solution's stability at room temperature and in a refrigerator (5°C3°C), the solutions were stored at room temperature and 5°C3°C.

Robustness

In accordance with the test method, standard and sample solutions loaded with known related substances at a specific concentration were prepared. In order to evaluate the suitability and stability of the system, these samples were injected into HPLC under various conditions. Changes in conditions include column oven temperature (± 5°C), buffer pH variation (± 0.2 unit), flow rate (± 10%), wavelength (± 2 nm), organic variation in mobile phase - A (2% final) and column (Batch / Lot variation) relative to the methodology values.

Forced Degradation

The specificity and stability of the devised method were demonstrated through the application of forced degradation. Baricitinib was subjected to hydrolysis (acid and base), photolysis, oxidation, heat, and humidity testing[8]. The drug was treated with 1M HCl at 70°C for 90 minutes for acid degradation, 1M NaOH for 50 minutes at room temperature (RT) for base degradation, and 0.1% H_2O_2 at RT for 20 minutes for peroxide degradation. The standard solution was also heated at 105 °C for 120 hours in order to evaluate heat degradation. To assess photolytic degradation, the medication was exposed to 200-watt hours per square meter of UV light and 1.2 million lux hours of White fluorescent light. In addition, the humidity degradation was analyzed for 120 hours at 92.5% RH and room temperature.

RESULTS AND DISCUSSION

Method Development

After various trials, changes were done to the Buffer, mobile phases AandB, and columns in order to develop a method for determining and validating the stability of Baricitinib and its related substances. The development results were included in Table-1.

Table-1: Development of Methods for Baricitinib

Column	Buffer	Mobile Phase	Conclusion
C18	1000 ml of water + 3.45 g of Ammonium dihydrogen orthophosphate. pH-3.5	Mobile Phase A: 90:10 v/v of degassed Buffer and Acetonitrile Mobile Phase B: Acetonitrile	The Barcitinib peak shape was not good, and the Barcitinib methyl ester was not separated, which was merged with the Barcitinib peak.
C18	1000 ml of water + 1.36g of potassium dihydrogen orthophosphate.	Mobile Phase A: Buffer Mobile Phase B: Acetonitrile	Less resolution of barcitinib and barcitinib methyl ester and blank interference was observed.
C18	1000 ml of water + 1.36g of potassium dihydrogen orthophosphate. pH-6	Mobile Phase A: 95:5 v/v of degassed Buffer and Acetonitrile Mobile Phase B: Acetonitrile	all peaks were well separated, but blank interference was observed. Hence, the column was changed from C18 to C8.

The final chromatographic conditions included a 4.6 mm internal diameter by 250 mm long inert-sustain C8 column filled with "Octyl silane chemically bonded to porous silica particles" (c8), with a flow rate of 1 ml per minute at UV detection of 225 nm and a column oven temperature of 45 °C at the end of a 50-minute data acquisition period. The gradient program for validating Baricitinib and related compounds is detailed in Table-2.

Table-2: Gradient Program for Baricitinib

Time (min)	Mobile Phase A (% v/v)	Mobile Phase B (% v/v)
0.01	82	18
15	65	35
35	35	65
50	35	65
52	82	18
60	82	18

Validation

The closely eluting peaks of Baricitinib-related substances were separated from the chromatograms of related substances and spiked samples. Peak purity data of known related compounds indicate that there are no co-eluting peaks and that the peaks are homogeneous in both the spiked and diluted samples of Baricitinib and the control substance. Based on the previously specified data, the test method is specific for identifying related compounds in the drug Baricitinib. The obtained retention time of Baricitinib is comparable to that of the standard, and the retention time (RT) of the control and spiked samples, as well as the relative retention time (RRT), purity angle, and threshold of spiked samples of Baricitinib and its relative substances, are listed in Tables-3 and 4. The chromatograms of these results are shown in Fig.-7 and 8.

Table-3: Retention Time of the Control Samples of Baricitinib and its Relative Substances

Name of the Control Sample	RT (min.)
Baricitinib carboxamide	6.384
Baricitinib	10.821
Baricitinib methyl ester	12.306
Pyrrolopyrimidine Baricitinib	14.591
Azetidinyl Baricitinib	19.606
Baricitinib methyl pivalate	26.768

Table-4: Other Parameters of the Spiked Samples of Baricitinib and its Relative Substances

Name of the spiked sample	RT (min.)	RRT	Purity angle	Purity threshold
Baricitinib carboxamide	6.330	0.59	0.334	1.479
Baricitinib	10.771	1.00	0.006	0.260
Baricitinib methyl ester	12.257	1.14	0.250	1.186
Pyrrolopyrimidine Baricitinib	14.546	1.35	0.113	0.945
Azetidinyl Baricitinib	19.576	1.82	0.739	2.301
Baricitinib methyl pivalate	26.767	2.49	0.792	2.075

*RT - Retention time; RRT- Relative retention time

LoD and LoQ

The data indicated that the signal-to-noise ratio for Baricitinib and its related substances fulfilled the acceptance criteria for LOD, LOQ, and % RSD[9]. Therefore, the developed procedure for quantifying related substances in Baricitinib is precise. In addition, the LOQ value for Baricitinib and Baricitinib-related substances was less than fifty percent of the specification level. Table-5 contains the statistical information for six replicates of Barnicitinib and its related substances.

Linearity

The correlation coefficient between Baricitinib and its known related substances was determined to be greater than 0.990. Consequently, the response of baricitinib and each related substance was linear from the LOD to 120% of the specific level. The results are listed in Table-6. The linearity diagram (concentration versus area) of Baricitinib and comparable substances is depicted in Fig.-9 to14.

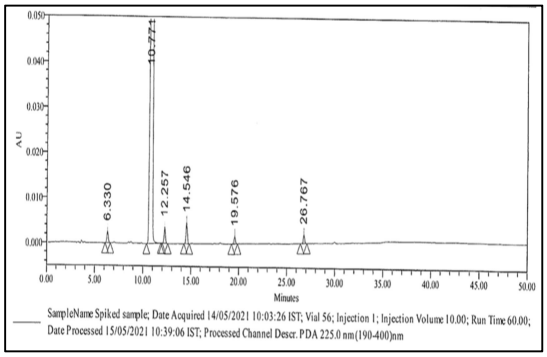


Fig.-7: Chromatogram Showing the Retention Time of the Control Samples of Barnicitinib and its Related Substances

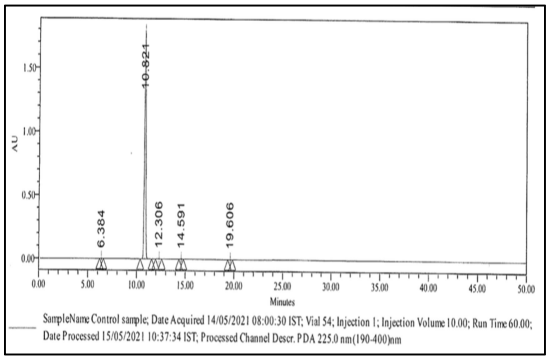


Fig.-8: Chromatogram Showing the Retention Time of the Spiked Samples of Barnicitinib and its Related Substances

Table-5: Lo D and Lo Q of the Barnicitinib and its Related Substances

Name of the sample	Area (Mean ± SD)		%RSD		Conc. (µg/mL)		Conc. (% w/w)		Signal-to-noise ratio	
	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ
Baricitinib	1751 ± 104	4682 ± 23	5.9	0.5	0.040	0.123	0.008	0.025	7	-
Baricitinib carboxamide	1424 ± 30	4311 ± 18	2.1	0.4	0.044	0.132	0.009	0.026	6	-
Baricitinib methyl ester	2046 ± 36	6099 ± 17	1.8	0.3	0.065	0.197	0.013	0.039	7	-
Pyrrolopyrimidine Baricitinib	2031 ± 36	5994 ± 32	1.8	0.5	0.043	0.131	0.009	0.026	7	-
Azetidinyl Baricitinib	1551 ± 21	4513 ± 14	1.4	0.3	0.065	0.197	0.013	0.039	5	-
Baricitinib methyl pivalate	1548 ± 12	4535 ± 36	0.8	0.8	0.055	0.166	0.011	0.033	5	-

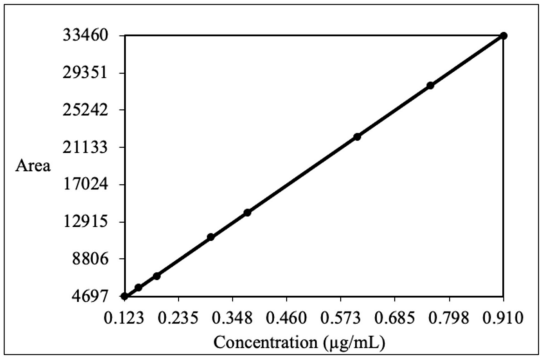


Fig.-9: Linearity Plot of Baricitinib

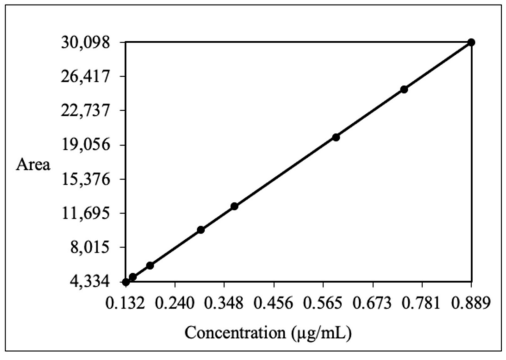


Fig.-10: Linearity Plot of Baricitinib Carboxamide

Table-6: Showing Linearity of Baricitinib and its Related Substances

Name of the sample	Slope	Intercept	Residual sum of squares	Correlation coefficient
Baricitinib	36724	71	43888	0.9999
Baricitinib carboxamide	33999	-183	21449	0.9999
Baricitinib methyl ester	30213	125	5838	0.9999
Pyrrolopyrimidine Baricitinib	45893	15	28107	0.9999
Azetidinyl Baricitinib	22740	81	9127	0.9999
Baricitinib methyl pivalate	27141	46	20664	0.9999

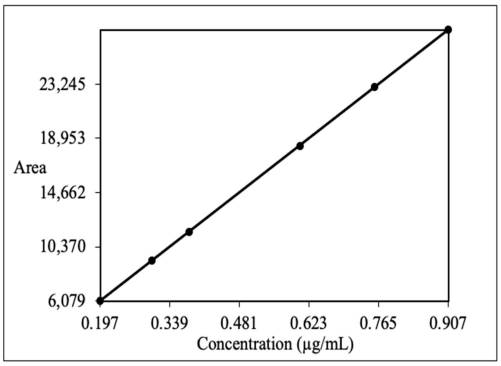


Fig.-11: Linearity Plot of Baricitinib Methyl Ester

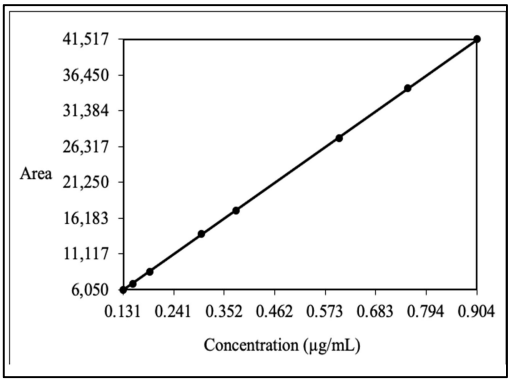


Fig.-12: Linearity Plot of Pyrrolopyrimidine Baricitinib

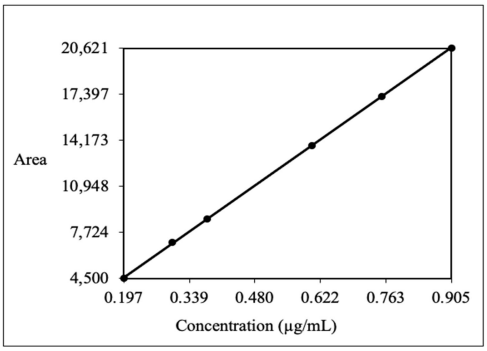


Fig.-13: Linearity plot of Azetidinyl Baricitinib

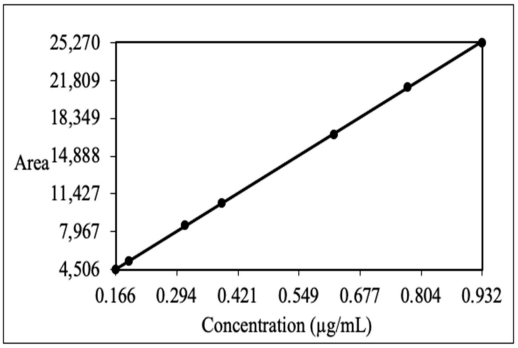


Fig.-14: Linearity Plot of Baricitinib Methyl Pivalate

Precision

The finding of Intermediate precision demonstrated that the devised method is robust for determining related compounds in Baricitinib drug substance despite differences in analyst, column, day, and system.[6] The outcomes are shown in Table-7.

Table-7: Showing Precision of Baricitinib and its Related Substances

Name of the sample	Method Precision			Intermediate Precision (Overall Precision)		
	%w/w (Mean ± SD)	% RSD	95% Confidence interval (±)	Area (Mean ± SD)	% RSD	95% Confidence interval (±)
Baricitinib carboxamide	0.158 ± 0.001	0.6	0.001	0.154 ± 0.005	3.2	0.003
Baricitinib methyl ester	0.248 ± 0.007	2.8	0.007	0.244 ± 0.007	2.9	0.004
Pyrrolopyrimidine Baricitinib	0.209 ± 0.001	0.5	0.001	0.209 ± 0.001	0.5	0.001
Azetidinyl Baricitinib	0.160 ± 0.001	0.6	0.001	0.158 ± 0.003	1.9	0.002
Baricitinib methyl pivalate	0.156 ± 0.001	0.6	0.001	0.154 ± 0.002	1.3	0.001

Accuracy

Based on the recovery results, the test method can accurately identify related compounds in Baricitinib drug substance at concentrations ranging from the LOD to 120% of the specification.[9] The results of accuracy at various levels are shown in Tables-8 and 9.

Table-8: Showing the Accuracy of Baricitinib and its Related Substances at the LOQ Level

Name of the sample	LOQ Level		
	%w/w (Mean ± SD)	% RSD	95% Confidence interval (±)
Baricitinib carboxamide	96.1 ± 0.46	0.5	1.1
Baricitinib methyl ester	96.9 ± 3.39	3.5	8.4

Pyrrolopyrimidine Baricitinib	94.4 ± 0.83	0.9	2.1
Azetidinyl Baricitinib	100.9 ± 0.81	0.8	2.0
Baricitinib methyl pivalate	103.5 ± 0.68	0.7	1.7

Table-9: Showing Accuracy of Baricitinib and its Related Substances at Different Specifications

Name of the sample	50% Level		100% Level		120% Level		Overall accuracy		
	%w/w (Mean ± SD)	% RSD	%w/w (Mean ± SD)	% RSD	%w/w (Mean ± SD)	% RSD	%w/w (Mean ± SD)	% RSD	95% Confidence interval (±)
Baricitinib carboxamide	101.8 ± 0.75	0.7	100.9 ± 0.40	0.4	101.3 ± 0.87	0.9	101.4 ± 0.72	0.7	0.6
Baricitinib methyl ester	103.6 ± 5.3	5.0	100.7 ± 0.70	0.7	100.0 ± 1.70	1.7	101.4 ± 3.19	3.1	2.5
Pyrrolopyrimidine Baricitinib	99.1 ± 0.75	0.8	99.1 ± 0.35	0.4	99.8 ± 1.15	1.2	99.3 ± 0.79	0.8	0.6
Azetidinyl Baricitinib	99.6 ± 1.50	1.5	99.8± 0.40	0.4	100.0 ± 0.60	0.6	99.8 ± 0.85	0.9	0.7
Baricitinib methyl pivalate	103.1 ± 2.04	2.0	102.0± 0.00	0.0	102.2 ± 1.10	1.1	102.4 ± 1.26	1.2	1.0

Robustness

The mobile phase and standard sample solution were found to be stable for up to 48 hours at both room temperature and a refrigerator (5°C3°F). Moreover, it was demonstrated that, in contrast to the STP condition, there is little variation in the RRTs of related compounds from the chromatograms of spiked samples of known related substances under the aforementioned robustness conditions. Thus, the test technique is reliable for identifying related compounds in the Baricitinib drug substance, regardless of the variations studied for each of the aforementioned parameters. These robustness results are detailed in Table- 10.

Table-10: Showing Robustness of the Baricitinib-Related Substances at Different Specification Levels

Parameter	Variation	RRT				
		Baricitinib carboxamide	Baricitinib methyl ester	Pyrrolopyrimidine Baricitinib	Azetidinyl Baricitinib	Baricitinib methyl pivalate
STEP	-	0.59	1.14	1.35	1.82	2.49
Flow rate	-10%	0.60	1.13	1.33	1.77	2.39
	+10%	0.58	1.14	1.37	1.86	2.58
STEP	-	0.58	1.13	1.34	1.80	2.41
Column lot variation STP**	-	0.60	1.14	1.34	1.76	2.41
STEP	-	0.59	1.14	1.35	1.82	2.49
Column oven temperature	-5°C	0.58	1.12	1.33	1.81	2.43
	+5°C	0.59	1.15	1.37	1.82	2.54
STEP	-	0.59	1.14	1.35	1.82	2.49
Wavelength variation	-2 nm	0.59	1.14	1.35	1.82	2.49
	+2 nm	0.59	1.14	1.35	1.82	2.49
STEP	-	0.58	1.14	1.35	1.81	2.50
Buffer pH	-0.2 units	0.58	1.14	1.35	1.80	2.47

variation	+0.2 units	0.58	1.14	1.35	1.81	2.49
STEP	-	0.60	1.14	1.34	1.76	2.41
Organic variation in mobile phase -A	-2% absolute	0.61	1.14	1.33	1.71	2.29
	+2% absolute	0.58	1.15	1.36	1.81	2.54

Forced Degradation

Baricitinib is susceptible to degradation under alkaline degradation conditions (1M NaOH / RT / 50 minutes), as evidenced by the results of various Baricitinib degradation tests conducted under various conditions. In contrast, it is stable under conditions of acidic degradation (1M HCl / 70°C / 90 minutes), oxidative degradation (0.1% H2O2 / 70°C / 20 minutes), thermal, photolytic, and humidity degradation. Based on the data generated by forced degradation, it is possible to conclude that baricitinib carboxamide is a potential impurity (Fig.-15).

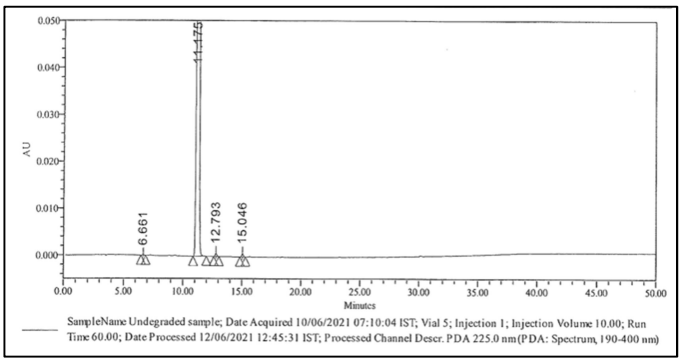
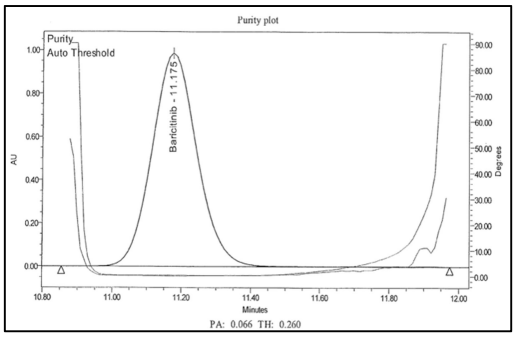
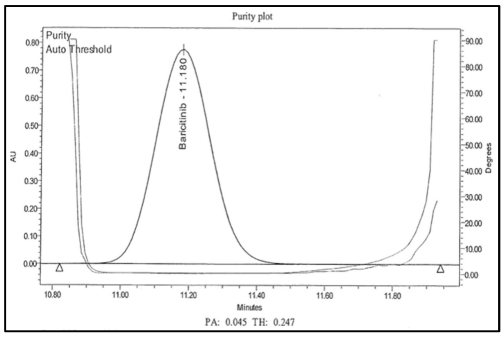


Fig.-15: Chromatogram of Baricitinib Drug Substance – Undegraded Sample (Acid, Base, and Peroxide)

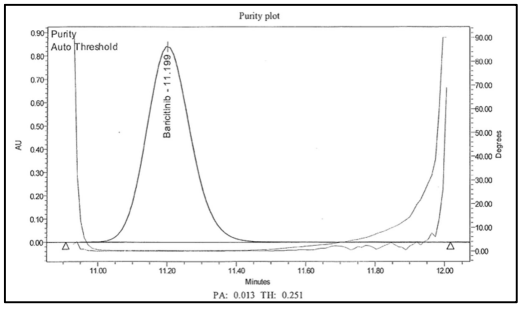
In addition, the peak purity data for each degradation sample's Baricitinib peak revealed that it is homogenous and does not co-elute with other peaks. Consequently, it was determined that the test method was specific and stable, allowing for the detection of specific/nonspecific Baricitinib-related substances (Fig.-16 a-h).



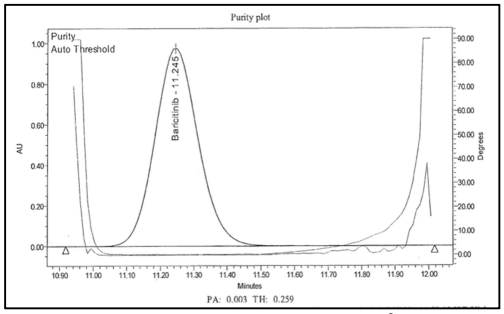
(a.)Undegraded Sample (Acid, base and peroxide)



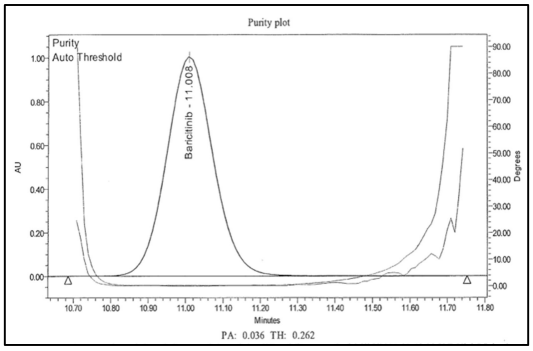
(b.)Undegraded Sample (Acid, base and peroxide)



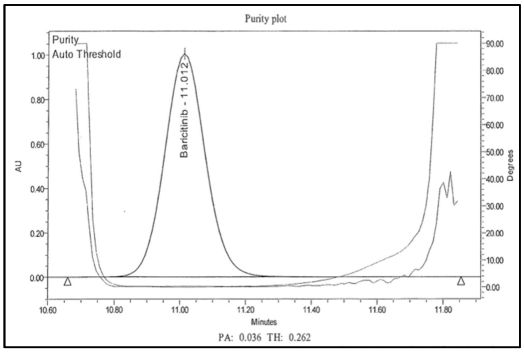
(c) Base Degradation (1M NaOH/RT/50 minutes)



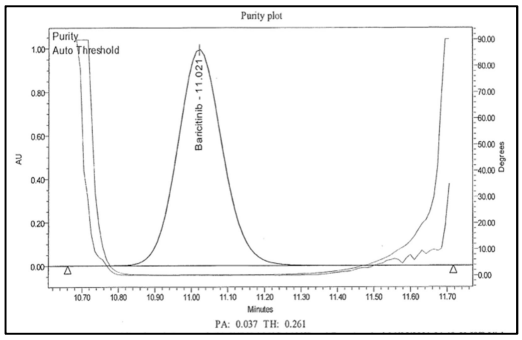
(d) Peroxide Degradation (0.1% H_2O_2/70°C/20 minutes)



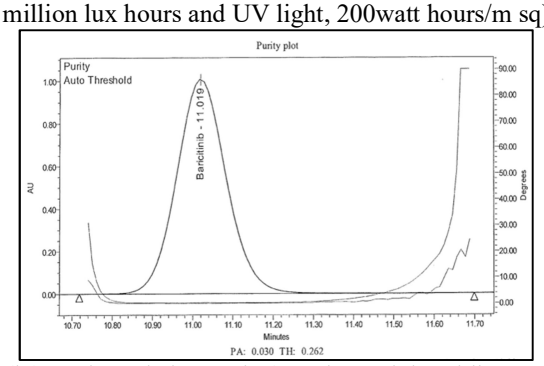
(e.) Thermal Degradation (105°C/120 hours)



(f.) Photolytic Degradation (white fluorescent light, 1.2 million lux hours and UV light, 200watt hours/m sq)



(g.) Humidity Degradation (92.5%RH/25°C/120 hours)



(h.) Undegraded Sample (For thermal, humidity and Photolytic degradation)

Fig.-16: Purity Plots of Baricitinib in Different Degradation Methods (a-h)

CONCLUSION

The developed approach is advantageous for identifying the drug baricitinib and its associated compounds since it is accurate, exact, specific, linear, stable, and sensitive. Appropriateness of the system, range, mobile phase stability, specificity, the limit of detection and limit of quantification, linearity, precision (system, method, and intermediate precision), and accuracy were approved for the test method. These study results have been determined to meet the established acceptance criteria. Based on the information provided, the developed test method for identifying related compounds in the Baricitinib drug substance is suitable and can be implemented in routine use.

CONFLICT OF INTERESTS

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

Gannamani Kiran Kumar https://orcid.org/0000-0003-3103-6287
Sudhakar Chintakula https://orcid.org/0000-0001-6441-2088

REFERENCES

1. A. Mogul, K. Corsi and L. McAuliffe. *Annals of Pharmacotherapy*, **53(9)**, 947(2019), https://doi.org/10.1177/1060028019839650

2. S. Assadiasl, Y. Fatahi, B. Mosharmovahed, B. Mohebbi and M. H. Nicknam. *The Journal of Clinical Pharmacology,* **61(10)**, 1274(2021), https://doi.org/10.1002/jcph.1874
3. F. Cantini, L. Niccoli, C. Nannini, D. Matarrese, M.E. Di Natale, P. Lotti, *et al.*, *Journal of Infection,* **81(4)**, 647(2020), https://doi.org/10.1016/j.jinf.2020.06.052
4. A.C. Kalil, T. F. Patterson, A.K. Mehta, K.M. Tomashek, C. R. Wolfe, V. Ghazaryan, *et al.*, *New England Journal of Medicine,* **384(9)**, 795(2021), https://doi.org/10.1056/NEJMoa2031994
5. Z. Lin, J. Niu, Y. Xu, L. Qin, J. Ding and L. Zhou. *Journal of Medical Virology*, **94 (4)**, 1523(2022), https://doi.org/10.1002/jmv.27482
6. M. MM and P.D. Hamrapurkar. *International Journal of Pharmaceutical Quality Assurance*, **12(1)**, 40(2021).
7. S. Veeraraghavan, S. Thappali, S. Vishwanadha, S. Vakkalanka, M. Rangaswamy, *Scientia Pharmaceutica,* **84(2)**, 347(2016), https://doi.org/10.3797/scipharm.1510-08
8. S. Gandhi, B. Kapoor, *Journal of Drug Delivery and Therapeutics,* **9(4-S)**, 488(2019), https://doi.org/10.22270/jddt.v9i4-s.3230
9. M. Seelam, S. Nathani, L. Kondraganti. *International Journal of Management and Humanities,* **4(2)**, 4 (2019), https://doi.org/10.35940/ijmh.A0369.104219
10. J.N. Rao, C. Sudhakar and S.S. Dubey. *Research Journal of Pharmacy and Technology,* **14(7)**, 3855(2021), https://doi.org/10.52711/0974-360X.2021.00668

[RJC-8344/2023]