ANALYTICAL METHOD DEVELOPMENT AND VALIDATION BY RP-HPLC METHOD FOR THE ESTIMATION OF UPADACITINIB BULK AND DOSAGE FORM

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ABSTRACT

Background:
Upadacitinib is a selective JAK1 inhibitor approved for rheumatoid arthritis, psoriatic arthritis, atopic dermatitis, and inflammatory bowel disease. It works by blocking the JAK-STAT signaling pathway, suppressing cytokine-driven inflammation. Despite its growing clinical use, published RP-HPLC methods for routine quality control of upadacitinib in tablet dosage forms remain limited. Accurate quantification in bulk and formulated products is essential for regulatory compliance under ICH, WHO, and FDA guidelines.

Objectives:
To develop a simple, sensitive, accurate, and economical RP-HPLC method for the estimation of upadacitinib in bulk drug substance and tablet dosage form (UPADOZ 30 mg), and to validate it according to ICH Q2(R1) guidelines.

Materials and Methods:
Upadacitinib bulk API (Vidisha Analytical) and UPADOZ 30 mg tablets (MSN Laboratories) were used. Chromatographic separation was performed on an Agilent 1260 Infinity II HPLC system using a Zorbax RX C18 column (150 × 4.6 mm, 5 µm). The mobile phase was Water: Acetonitrile (40:60 v/v), delivered at 1.0 mL/min with UV detection at 232 nm. Column temperature was maintained at 40°C and injection volume was 20 µL. Drug identity was confirmed by FTIR and UV spectroscopy prior to chromatographic analysis. Method validation covered system suitability, specificity, linearity, accuracy, precision (method and intermediate), and robustness per ICH guidelines.

Results:
The method yielded a retention time of 3.37 minutes with a tailing factor of 1.40 and theoretical plates of 5635, all within acceptance criteria. Linearity was established from 30 to 90 ppm ($r^2 = 1.000$). Accuracy at 50%, 100%, and 150% levels gave mean recoveries of 100.22%, 100.36%, and 99.70%, respectively. Method precision showed %RSD of 0.415%, and intermediate precision %RSD was 0.5%. Blank and placebo showed no interference at the upadacitinib retention time. Robustness studies with deliberate changes in flow rate (±0.1 mL/min) and wavelength (±2 nm) met all system suitability criteria.

Conclusion:
The developed RP-HPLC method is accurate, precise, specific, reproducible, and robust for routine estimation of upadacitinib in bulk and tablet formulation. It meets ICH validation requirements and can be reliably applied in pharmaceutical quality control laboratories.

Keywords: Upadacitinib, RP-HPLC, Method development, Method validation, ICH guidelines, JAK1 inhibitor, Tablet dosage form, Quality control.

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1. INTRODUCTION

Analytical chemistry forms the backbone of pharmaceutical quality control, providing the tools necessary to characterize, quantify, and monitor active pharmaceutical ingredients (APIs) in both bulk and finished dosage forms [1]. At its core, it encompasses qualitative and quantitative methods identifying what is present in a sample and precisely determining how much. In drug development and manufacturing, this distinction carries real regulatory weight: product quality cannot be assumed; it must be demonstrated through rigorous, validated measurement.

Chromatographic techniques have become the standard workhorse for pharmaceutical analysis. Among these, Reverse Phase High Performance Liquid Chromatography (RP-HPLC) is widely preferred owing to its sensitivity, reproducibility, and adaptability across a broad range of drug molecules [2]. The method separates analytes based on hydrophobic interactions between the sample components and a nonpolar stationary phase, with a polar mobile phase driving the elution. Because most pharmaceutically relevant compounds fall into this analytical window, RP-HPLC remains the method of choice in quality control laboratories worldwide.

Analytical method development, however, is not a straightforward exercise. It demands careful selection of chromatographic conditions mobile phase composition, flow rate, pH, column type, detection wavelength followed by systematic optimization and, critically, formal validation. The ICH Q2 (R1) guidelines define the framework for this validation, requiring demonstration of specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ) before any method can be accepted for routine use [3]. Without this evidence, even a seemingly functional method offers no assurance of reliability across laboratories, analysts, or time.

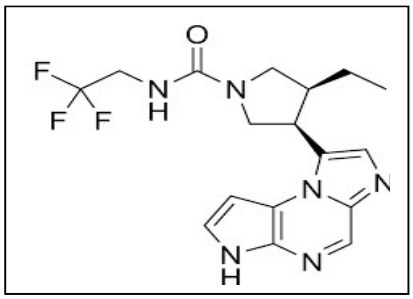


Fig: 1 Structure of Upadacitinib.

Upadacitinib is a second-generation, selective JAK1 inhibitor developed by AbbVie and marketed as RINVOQ. It is approved for the treatment of rheumatoid arthritis, psoriatic arthritis, atopic dermatitis, ankylosing spondylitis, and related inflammatory conditions [4]. Its selectivity for JAK1 over JAK2 (74-fold), JAK3 (58-fold), and TYK2 represents a meaningful improvement over first-generation jakinibs such as tofacitinib and ruxolitinib, which lacked subtype specificity and produced dose-limiting side effects [5]. Mechanistically, upadacitinib blocks JAK1-mediated cytokine signaling through the JAK-STAT pathway, thereby reducing the inflammatory cascade driving autoimmune pathology.

Pharmacokinetically, upadacitinib is orally bioavailable, reaching peak plasma concentrations within two to four hours of ingestion. It is primarily metabolized by CYP3A4, with 52% plasma protein binding and a terminal half-life of 9 to 14 hours [6]. These properties make it amenable to once-daily extended-release tablet formulations, such as the 30 mg UPADOZ tablet used in the current study.

Despite the drug's clinical adoption, published RP-HPLC methods for upadacitinib in bulk and solid dosage forms remain limited. Existing reports have employed LC-MS/MS for bioanalytical applications in biological matrices [7] and stability-indicating methods focused on degradation product profiling [8], but simple, validated RP-HPLC assay methods for routine pharmaceutical QC are still scarce. This gap is what the present work addresses. The aim of this study was to develop and validate a precise, accurate, and economical RP-HPLC method for the estimation of upadacitinib in bulk drug and tablet dosage form, following ICH Q2 (R1) guidelines, to support quality control applications in the pharmaceutical industry.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Upadacitinib working standard was procured from Vidisha Analytical, while the commercial tablet formulation (UPADOZ 30, upadacitinib 30 mg extended-release tablets, MSN Laboratories) served as the dosage form sample. Acetonitrile and water of HPLC grade were used throughout. All reagents and solvents were of analytical or HPLC grade and used without further purification.

2.2 Instrumentation

Chromatographic analysis was performed on an Agilent 1260 Infinity II HPLC system equipped with a UV detector, operated via Open Lab EZ Chrome workstation software. UV absorbance scanning for wavelength selection was done on a Jasco UV 550 double-beam spectrophotometer with matched 10 mm quartz cells, using Spectra Manager software. An Aczet CY224C analytical balance (range: 2 mg

to 200 g) was used for all weighing operations. A digital pH meter was used to verify mobile phase pH where required.

2.3 Chromatographic Conditions

Separation was carried out on a Zorbax RX C18 column (150 mm × 4.6 mm, 5 µm particle size) maintained at 40°C. The mobile phase consisted of water and acetonitrile in a ratio of 40:60 (v/v), delivered isocratically at a flow rate of 1.0 mL/min. Detection was set at 232 nm. Injection volume was 20 µL and the total run time was 7.0 minutes. Sample compartment temperature was maintained at 25°C. Seal wash was water: acetonitrile (90:10 v/v) and needle wash was water: acetonitrile (10:90 v/v).

2.4 Preparation of Mobile phase:

Prepare mixture of Water, Acetonitrile in the ratio of 40:60 v/v respectively, mix well. Sonicated for 15 min to degas the mobile phase.

2.5 Preparation of Diluent:

Prepare mixture of water and Acetonitrile in the ratio of 20:80 v/v respectively, mix well.

2.6 Preparation of Standard solution:
Weighed and transferred accurately about 50 mg of Upadacitinib working standard into 100 mL clean and dry volumetric flask. Added about 80 mL of diluent, sonicate to about 30 minutes to dissolve and dilute up to the mark with diluent and mix. Further dilute above stock 6.0 mL of this stock solution to 50 mL with diluent and mix well. Filter the sample solution through 0.45µ membrane Nylon filter. Discard first 2.0 mL of filtrate and then collected the sample.

2.7 Preparation of Sample solution:
Determine the average weight of 10 tablets and crush into fine powder. Weigh and transfer fine powder equivalent to 150 mg of Upadacitinib (1100 mg crush powder) into 500 mL volumetric flask. Stir the solution for 30 min with 500 RPM then sonicate for 30 min. with intermediate shaking. Allow to cool to room temperature. Dilute up to the mark with diluent and mix. Filter the sample solution through 0.45µ Nylon membrane syringe filter. Further diluted above filtered stock solution 5.0 mL of this sample stock solution to 25 mL volumetric flask make up with Diluent and mixed well. **(Concentration of Sample Solution: 60 ppm)**

3. RESULTS AND DISCUSSION

3.1 Drug Characterisation

3.1.1 Physical Characteristics

Upadacitinib was obtained as a white to off-white crystalline solid or fine powder, with no distinct or strong odour. The observed melting point is 153°C but it depends slightly on the specific crystalline form, typically ranging between 152-156°C.

3.1.2 Solubility Studies

Solubility studies were performed in various solvents commonly employed during chromatographic method development to evaluate the solubility characteristics of Upadacitinib. The drug was tested at a concentration of 3 mg/mL in Water, Methanol, Acetonitrile, and Water: Acetonitrile (20:80 v/v). Upadacitinib exhibited limited solubility in Water, while improved solubility was observed in Methanol and Acetonitrile. Complete and clear dissolution was achieved in the Water: Acetonitrile (20:80 v/v) mixture after sonication for 5–10 minutes, resulting in a stable solution suitable for chromatographic analysis. Therefore, the optimized mobile phase composition was selected as the diluent for all subsequent standard and sample preparations.

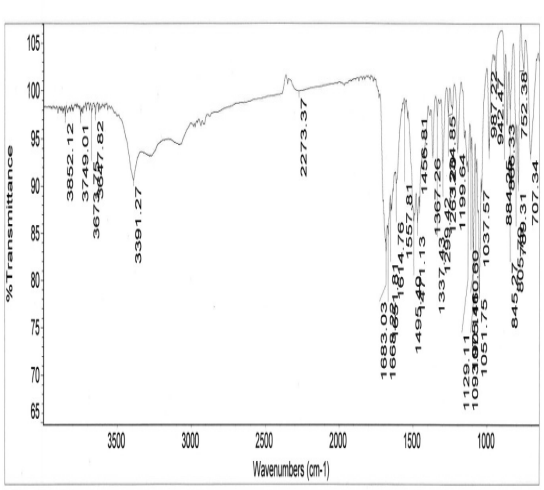
3.2 Infrared Spectroscopy

FTIR spectroscopy was performed to confirm the functional groups of Upadacitinib. The IR spectrum revealed characteristic absorption bands: N–H stretching at 3391.27 cm^{-1}, C=N stretching at 1683.03 cm^{-1}, C=O stretching at 1668.22 cm^{-1}, C=C stretching at 1614.76 cm^{-1}, C–N stretching at 1337.43 cm^{-1}, and C–F stretching at 1051.75 cm^{-1}. All observed peaks were in agreement with the standard IR ranges for respective functional groups, confirming the chemical identity of the drug. Table 1 and Figure 1.

Table 1: - IR peak Assignment value of Upadacitinib

Standard IR Ranges (cm^{-1})	IR Ranges (cm^{-1})	Functional Group
3400-3300	3391.27	N-H Stretching
1690-1640	1683.03	C=N Stretching
1685-1666	1668.22	C=O Stretching
1620-1610	1614.76	C=C Stretching
1342-1266	1337.43	C-N Stretching
1400-1000	1051.75	C-F Stretching

Fig 1: - FTIR spectrum of Upadacitinib.



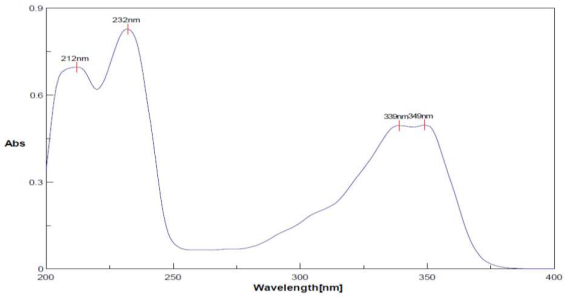
3.3 UV Spectroscopy and Wavelength Selection

UV spectroscopy was carried out by scanning the standard solution between 200–400 nm. Upadacitinib showed maximum absorbance (λmax) at 232 nm with an absorbance of 0.8123 AU. The blank showed no absorbance at this wavelength. Based on this, 232 nm was fixed as the analytical wavelength for all subsequent HPLC analysis. Solubility screening identified Water: Acetonitrile (20:80 v/v) as the suitable diluent, as the drug was insoluble in water and methanol alone but dissolved completely in acetonitrile and the water–acetonitrile mixture. Table 2 and Figure 2(a)

Table 2: - Determination of λ max of Upadacitinib

Sr. No.	Wavelength (nm)	Absorbance
1.	232	0.8123 A^0

.**Figure 2: - UV spectrum of Upadacitinib.**



3.4 Method Development and Optimisation

Six trials were conducted systematically to optimize the chromatographic separation. Trial 1 used a Peerless CN column (100 × 4.6 mm, 5µ) with Water: Methanol (90:10 v/v) at 1.5 mL/min elution occurred at 2.3 min but asymmetry was 0.73 with only 1250 theoretical plates, with broad and multiple peaks rejected. Trial 2, on the same CN column with Water: Methanol (70:30 v/v), gave elution at 1.9 min, asymmetry 0.82, 1563 plates; broad peak persisted rejected. Trial 3 substituted acetonitrile (Water: ACN 70:30 v/v, CN column), elution at 1.8 min, asymmetry 0.85, 1630 plates, with peak splitting at the apex due to high concentration rejected. Trial 4 switched to a Zorbax C18 column (150 × 4.6 mm, 5µ) with Water: ACN (70:30 v/v) at 1.0 mL/min elution at 2.50 min with asymmetry 2.36 and 1852 plates, but significant fronting was observed rejected. Trial 5, on the same C18 column with Water: ACN (50:50 v/v), gave elution at 3.80 min with improved asymmetry (1.32) and 3652 plates, but retention time and economic feasibility needed further optimization.

In Trial 6 the finalized method mobile phase was set to Water: Acetonitrile (40:60 v/v) on a Zorbax C18 (150 × 4.6 mm, 5µ) column, flow rate 1.0 mL/min, wavelength 232 nm, column temperature 40°C, injection volume 20 µL, run time 7.0 min. Upadacitinib eluted sharply at 3.37 min with tailing factor 1.40 and 5635 theoretical plates both within ICH acceptance criteria. This trial was selected for validation. Figure 3.

Table 3: Final optimized RP-HPLC chromatographic conditions column, mobile phase, flow rate, detection wavelength, injection volume, column temperature, and run time.

Chromatographic Conditions:

Column	Zorbax C18 (150 x 4.6mm) 5µ
Mobile Phase	Water: Acetonitrile (40:60 v/v)
Flow Rate	1 mL/min
Injection Volume	20 µL
Wavelength	232 nm
Column Temp.	40°C

Auto sampler Temp.	25°C
Run time	7.0 min.
Needle wash	Water : Acetonitrile (10:90 v/v)
Seal wash	Water : Acetonitrile (90:10 v/v)

Table 3: Final optimized RP-HPLC chromatographic conditions

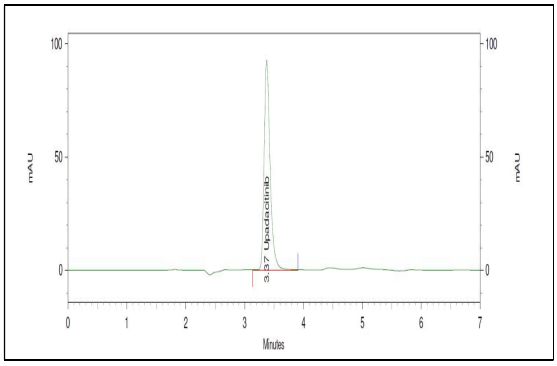


Fig 3: Typical chromatogram for Trial- 6

3.5 Method Validation

3.5.1 System Suitability

System suitability was evaluated using five replicate injections of the standard solution. The mean retention time of Upadacitinib was 3.37 min. The percentage relative standard deviation (%RSD) of peak areas was 0.09 %, which was well within the acceptance criterion of not more than 2.0%.

The tailing factor was 1.40 and the theoretical plate count was 5635, exceeding the minimum requirement of 2000 plates. These results demonstrated that the chromatographic system was suitable for routine analysis. (Table 3)

Table 4: System suitability parameters for Upadacitinib %RSD of peak area, tailing factor, theoretical plates, and resolution (n = 5 replicate injections)

Tailing Factor	**1.40**
Theoretical plates	**5635**
Injection No.	**Area**
1	10845203
2	10842514
3	10856538
4	10850129
5	10865938
Mean	**10852064**
%RSD	**0.09**

3.5.2 Specificity

The specificity is the ability to assess unequivocally the analyte of interest in the presence of component that may be expected to be present, such as impurities, degradation products, and matrix components.

Acceptance criteria: Purity Angle < Purity Threshold. No peak in blank/placebo at analyte RT

Specificity was evaluated by comparing chromatograms obtained from blank, standard, and sample solutions. No interfering peaks were observed at the retention time of Upadacitinib in the blank chromatogram. The retention time of Upadacitinib remained consistent across all injections at approximately 3.37 min. (Table 5 and Figure 4–7)

Table 5: Specificity data retention times for identification, blank interference results, and PDA peak purity angles and thresholds for Upadacitinib in standard and sample solutions.

Solution	**Specificity data**		
	Retention time (min)	**Purity Match**	
Blank solution	NA	NA	
Placebo solution	NA	NA	
Standard solution	3.37	Purity angle	Purity threshold
		0.68	1.25
	3.37	0.65	1.17

Fig 4: Chromatogram of Blank

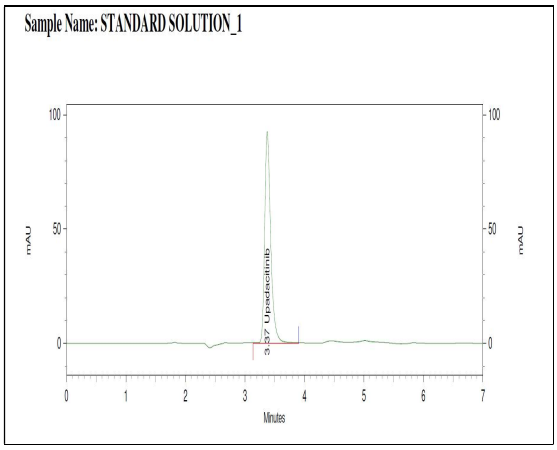


Fig 5: Chromatogram of Standard

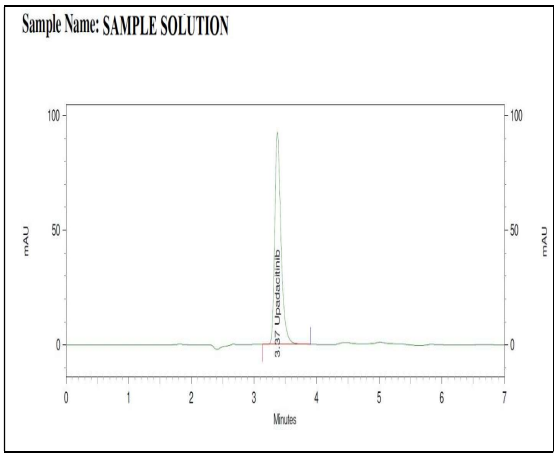


Fig 6: Chromatogram of Sample

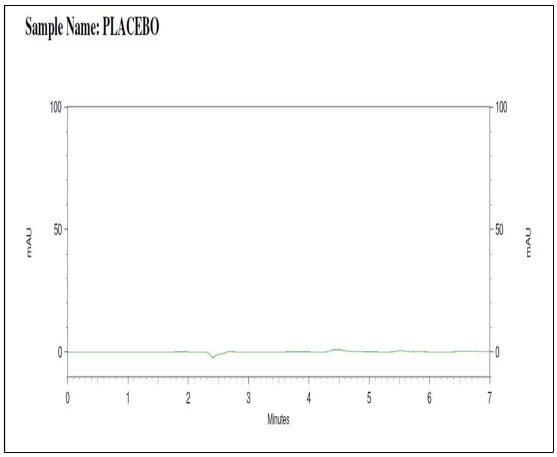


Fig 7: Chromatogram of Placebo

3.5.3 Linearity

Linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity is generally reported as the correlation coefficients, the slope of regression line i.e., $r^2 \geq 0.999$.

Linearity was evaluated in the range of 50 % to 150 % of Upadacitinib for working concentration. The working concentration of Upadacitinib in solution is 60 µg/mL.

Table 6: Linearity data for Upadacitinib concentration levels (60 µg/mL), peak areas, calibration curve parameters (slope, intercept, correlation coefficient r, % Y-intercept, RSS).

Level	Conc (µg/mL)	Area	Mean
50%	30	5430727	5440129
		5469531	
		5420129	
75%	45	8097771	8096251
		8092531	
		8098452	
100 %	60	10835632	10834760
		10842109	
		10826539	
125 %	75	13554558	13547749
		13520126	
		13568564	
150 %	90	16256430	16246034
		16232103	
		16249568	
Corr. Coeff			1.000
Intercept			7661.4
Slope			180422.053
% Y-intercept			0.07

Table 6: Linearity of Upadacitinib

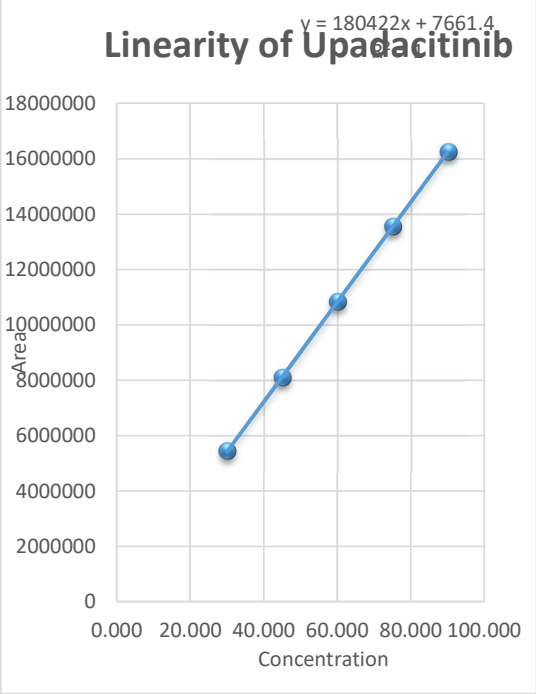


Fig 8: Linearity plot of Upadacitinib

3.5.4 Accuracy

The accuracy of an analytical procedure as the closeness of agreement between the conventional true value or an accepted reference value and the value found. Evaluated accuracy from 50% to 150% of Upadacitinib tablet, working concentration level. Each level prepared in triplicates.

The mean percentage recoveries obtained were 100.22%, 100.36%, and 99.70% at the respective levels. The data shows that the Mean recovery for 50% to 150% is in the range of 98.0%-102.0% and individual recovery for 50% to 150% is in the range of 95.0% - 105.0% in table no 7.

Table 7: % Recovery for Upadacitinib

Level (%)	Upadacitinib Added Conc (µg/mL)	Upadacitinib Recovered conc	Area	% Recovery	Mean % Recovery
50	30.04	30.04	5469532	100.00	100.22
	30.15	30.06	5492101	99.62	
	29.86	30.07	5503	101.02	
			544		
100	60.10	59.96	10809568	99.41	100.36
	60.02	60.13	10952521	100.32	
	59.85	60.20	10975362	101.34	
150	89.98	89.90	16253265	99.72	99.70
	89.92	90.08	16342125	100.66	
	90.05	89.68	16135867	98.73	

3.5.5 Precision

3.5.5.1 Method Precision:

Method precision was assessed using six independently prepared sample solutions. The mean assay value obtained was 99.89% with a %RSD of 0.134%, indicating excellent repeatability.

Single injection of blank (Diluent), Standard solution (five replicates) and sample solution (six preparations) was injected on the system. (Table 8)

Sample	**Area**	**% Assay**
Sample 1	10853542	100.05
Sample 2	10832749	99.83
Sample 3	10828569	99.77
Sample 4	10841253	99.98
Sample 5	10829568	99.71
Sample 6	10851233	99.97
Mean		**99.89**
STD DEV		**0.1343**
% RSD		**0.134**

Table 8: Method precision on Bulk

Sample	Area	% Assay
Sample 1	10653226	98.20
Sample 2	10685421	98.48
Sample 3	10712534	98.70
Sample 4	10685421	98.55
Sample 5	10595635	97.56
Sample 6	10655236	98.16
Mean		**98.27**
STD DEV		**0.4081**
% RSD		**0.415**

Table 9: Method precision on Pharmaceutical dosage form

Conclusion:

- The data shows that system suitability is fulfilled.
- The data shows that % RSD for % Assay is within the acceptance criteria and hence the method is precise.

3.5.5.2 Intermediate Precision:

Six independent sample preparations were prepared on different day and by different analyst and injected on the HPLC. The mean assay value obtained was 98.36% with a %RSD of 0.629%.

Table 10: Intermediate Precision

Sample	Area	% Assay
Sample 1	10742102	98.97
Sample 2	10682155	98.47
Sample 3	10745128	99.03
Sample 4	10678424	98.45
Sample 5	10598655	97.59
Sample 6	10588444	97.66
Mean		**98.36**
STD DEV		**0.6183**
% RSD		**0.629**

Table 11: Intermediate Precision pool Data

Parameter	Method Precision	Intermediate Precision
	(Analyst-I)	**(Analyst-II)**
HPLC NO.	**AD/HPLC-02**	**AD/HPLC-04**
Column No.	**HPLC-86**	**HPLC-62**
Sample No.	**%Assay**	
1	98.20	98.97
2	98.48	98.47
3	98.70	99.03
4	98.55	98.45
5	97.56	97.59
6	98.16	97.66
Mean	**98.27**	**98.36**
Mean of Precision % Assay	**98.32**	
Absolute Mean difference % assay	**0.5**	

Conclusion:

- The data shows that system suitability is fulfilled.
- The data shows that % Assay is of six samples is not more than 2.0
- The data shows that % Assay is within the acceptance criteria and hence the method is rugged

3.5.6 Robustness

Robustness of an analytical procedure as a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. This parameter was studied by making small, deliberate changes in the chromatographic conditions and Assay parameters, observing the effect of these changes on the system suitability and results obtained by injecting the standard and sample solutions.
Flow rate variation to 0.9 mL/min and 1.1 mL/min resulted in assay values of 99.6% and 98.3%, respectively. Similarly, wavelength variations to 234 nm and 230 nm produced assay values of 99.8% and 97.4%, respectively. All results remained within acceptable limits, and system suitability parameters were maintained under each modified condition.

Table 11: Robustness data for Upadacitinib % assay and system suitability parameters under deliberate variations in flow rate (±0.1 mL/min), detection wavelength (±2 nm), and column temperature (±2°C), with absolute difference from control condition.

Change in parameter	Condition	Area	% Assay	Absolute difference of % Assay
Control	As per method	10653226	98.0	NA
Change in flow rate1.0 ml/min (±0.1 ml/min)	1.1 ml/min	10691363	98.3	0.3
	0.9 ml/min	10831292	99.6	1.6
Change in wavelength (±2 nm)	234 nm	10852533	99.8	1.8
	230 nm	10595322	97.4	0.6

Table 9.12 Robustness for Upadacitinib

4. DISCUSSION

Developing a reliable RP-HPLC method for Upadacitinib required systematic screening across six chromatographic trials before a suitable set of conditions emerged. The early trials on a Peerless CN column with methanol-based mobile phases consistently failed broad peaks, poor asymmetry values below 1.0, and theoretical plate counts under 1600 indicated inadequate retention and column-analyte compatibility. Switching the organic modifier from methanol to acetonitrile in Trial 3 did not resolve the issue either; peak splitting at the apex suggested column overloading at the tested injection volume of 50 µL on the CN stationary phase. The shift to a Zorbax C18 column in Trial 4 was the turning point. C18 chemistry offered better hydrophobic interaction with Upadacitinib, a moderately lipophilic JAK1 inhibitor, and elution at 2.50 min with Water: ACN (70:30 v/v) showed improved plate count (1852), though significant fronting remained. Reducing the aqueous proportion progressively 50:50 in Trial 5 and then 40:60 in Trial 6 increased organic content in the mobile phase, which shortened and sharpened the peak. Trial 6 produced the cleanest result: retention time 3.37 min, tailing factor 1.40, and 5635 theoretical plates. The reduction in injection volume from 50 µL to 20 µL between trials also contributed to the improvement by reducing peak distortion from volume overload. The final conditions Water: ACN (40:60 v/v), 1.0 mL/min, 232 nm, column temperature 40°C were selected for validation. The wavelength of 232 nm, identified during UV scanning, aligned with the λmax of Upadacitinib and offered adequate sensitivity at the working concentration of 60 µg/mL. FTIR characterization confirmed all key functional groups of the drug: the N–H stretch at 3391 cm^{-1}, the C=O and C=N carbonyl and imine absorptions around 1668–1683 cm^{-1}, and the C–F stretch at 1051 cm^{-1} all consistent with the known structure of Upadacitinib. These preliminary characterization steps established that the drug substance was authentic before method validation began. System suitability data from five replicate injections confirmed the method's readiness for routine use: %RSD of 0.09% is exceptionally low and indicates minimal run-to-run variability under the finalized conditions. Specificity results showed no interfering peaks from blank or placebo at the retention time of 3.37 min, and peak purity angles for both standard and sample were below their respective purity thresholds confirming the method selectively measures Upadacitinib without matrix interference. Linearity across 30–90 µg/mL (50–150% of working concentration) gave a correlation coefficient of 1.000 with a y-intercept contribution of just 0.07% of the standard response, which is well within the NMT 2.0% limit. This near-perfect linearity confirms proportional detector response across the validated range. Accuracy recoveries at three concentration levels averaged between 99.70% and 100.36% tight clustering around 100% that reflects minimal systematic error in the sample preparation procedure. Precision results were equally strong. Method precision on bulk (%RSD 0.134%) and on tablet dosage form (%RSD 0.415%) both fell far below the 2.0% threshold. The intermediate precision study conducted on a different instrument, by a different analyst, on a different day gave an absolute mean difference of only 0.5% between the two analysts, confirming the method is rugged across laboratory variables. Robustness testing showed that deliberate changes in flow rate (±0.1 mL/min) and detection wavelength (±2 nm) produced assay deviations of no more than 1.8%, within the ±5.0% acceptance limit. This demonstrates the method tolerates minor operational fluctuations without compromising results a practical necessity for any method intended for routine quality control use. Taken

together, the validated RP-HPLC method is accurate, precise, specific, linear, and robust. It offers a simple, economic, and reproducible approach for the routine estimation of Upadacitinib in both bulk drug substance and tablet formulation, and meets all ICH Q2 (R1) validation requirements.

5. CONCLUSION

A simple, precise, and economical RP-HPLC method was successfully developed and validated for the estimation of Upadacitinib in bulk drug substance and tablet dosage form. The method used a Zorbax C18 column (150 × 4.6 mm, 5µ) with Water: Acetonitrile (40:60 v/v) as mobile phase, UV detection at 232 nm, and a flow rate of 1.0 mL/min conditions arrived at after systematic optimization across six chromatographic trials. Validation carried out as per ICH Q2 (R1) guidelines confirmed the method meets all required performance criteria. Linearity was established over 30–90 µg/mL with a correlation coefficient of 1.000. Accuracy recoveries ranged from 99.70% to 100.36% across 50–150% concentration levels. Method precision gave %RSD below 0.42% on both bulk and dosage form, and intermediate precision showed an absolute mean difference of only 0.5% between two independent analysts confirming ruggedness. The method remained unaffected by small deliberate changes in flow rate and wavelength, demonstrating adequate robustness for routine laboratory use. No interference from excipients or matrix components was observed, establishing specificity. The method is sensitive, reproducible, and rapid, making it practically suited for quality control laboratories handling routine Upadacitinib analysis. Its straightforward sample preparation and short run time of 7.0 min add to its economic value. Going forward, this validated method can be extended to stability-indicating studies, pharmacokinetic investigations in biological matrices such as plasma and urine, and compatibility studies during new dosage form development including extended-release tablets and liquid formulations. It also holds potential for use in regulatory submissions and generic drug development programs seeking bioequivalence data for Upadacitinib.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest associated with this research work. This study was conducted purely for academic and scientific purposes as part of a postgraduate research programme. No financial support, funding, grants, or sponsorship was received from any pharmaceutical company, commercial organization, or any other external body that could have influenced the design, conduct, interpretation, or reporting of this research. The drug substance Upadacitinib used in this study was obtained solely for research purposes, and the findings reported here represent an independent academic investigation. No proprietary interest exists in any of the materials, methods, or results described in this manuscript. All data presented are original, transparent, and free from any bias arising out of commercial or personal interest.

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