RESEARCH ARTICLE

Development, Validation, and Forced Degradation Evaluation of a Green Stability-indicating RP-HPLC method for Upadacitinib in Bulk and Tablet Dosage Forms

Sai Prudhvi N[1], Raghavendra Kumar Gunda[2*], Lakshmi Swarupa Ch[3], Rani R[3], Akhila T[4], Meghana K4, Sharon P[4], Venkata Sri Kavya S[4]

[1]Associate Professor, Department of Pharmaceutical Analysis, Victoria College of Pharmacy, Nallapadu, Guntur (Dt), Andhra Pradesh, India-522005.

[2]Associate Professor, Department of Pharmaceutics, Narasaraopeta Institute of Pharmaceutical Sciences (Autonomous), Narasaraopet, Palnadu (Dt), Andhra Pradesh, India-522601.

[3]Associate Professor, Department of Pharmaceutics, Victoria College of Pharmacy, Nallapadu, Guntur (Dt), Andhra Pradesh, India-522005.

[4]Research Scholar, Department of Pharmaceutical Analysis, Victoria College of Pharmacy, Nallapadu, Guntur (Dt), Andhra Pradesh, India-522005.

Corresponding Author Email ID: raghav.gunda@gmail.com

Received: 03/05/2026 **Accepted:** 18/05/2026 **Published:** 15/07/2026

Abstract

Objective: The current study was to develop a simple, rapid, and reliable RP-HPLC method was proposed for the quantification of Upadacitinib in bulk drug and pharmaceutical dosage forms.

Materials & Methods: Separation was achieved on C18 column using ethanol and 10 mM ammonium acetate in 60:40 (v/v) at isocratic flow rate of 0.5 mL/min over 6 min runtime. The method produces sharp and well-resolved peak with 2.8 min retention time. It displays excellent specificity, with no interference from excipients.

Results: System suitability results were within acceptable limits, with tailing factor of 1.03 and 5813 theoretical plates indicates good column efficiency. The method demonstrates high sensitivity, with LOD and LOQ values of 0.025 µg/mL and 0.082 µg/mL, respectively. A strong linear response was noticed over 30-105 µg/mL (r^2 = 0.9999). Precision was proved with %RSD values of 0.49 (intra-day) and 0.60 (inter-day), while accuracy was observed in the range of 99.38 % to 100.47%. The method remains stable under small deliberate variations proves its robustness and ruggedness. The forced degradation studies show highest degradation under peroxide (8.76%), and acidic (4.75%), while drug remains stable under basic (3.81%) conditions, thermal (5.07%) and photolytic (2.12%) stress. No interference from degradation products was observed proves its stability-indicating capability. The assay was noticed to be 99.12 % and demonstrates suitability for routine quality and stability assessment of Upadacitinib. The sustainability of the method was evaluated with the use of the AGREE (0.82), and GAPI (4.8E+02), displayed in the center corresponds to the E-factor of 475, meaning that approximately 475 g of waste is generated per gram of analyte analyzed.

Conclusion: Overall, the results demonstrate the ability of the method to maintain a good balance between analysis reliability, environmental effects, and operational convenience.

Keywords: Upadacitinib, HPLC analytical method, Stability-indicating analysis, Method validation, formulation assay, greenness assessment

Journal of Applied Pharmaceutical Sciences and Research, (2026); DOI: 10.31069/japsr.v9i2.05

Introduction

The active pharmaceutical ingredient (API) was the key component in drug product responsible to produce intended therapeutic effect.[1] The accurate assessment of identity and quantity of API was very essential in pharmaceutical analysis because it was directly influences safety and efficacy of medicine. Among the available analytical techniques, RP-HPLC was widely used for this purpose.[2] This method enables effective separation, detection, and measurement of APIs in complex formulations. Owing to its high sensitivity, reliability, and suitability for broad range of compounds, RP-HPLC becomes an important tool in pharmaceutical research and routine quality control analysis.[3]

Upadacitinib is an orally bioavailable, selective Janus kinase (JAK) inhibitor that selectively targets JAK1-mediated signaling pathways that are involved in inflammatory and immune-mediated processes. It was developed with the aim of creating JAK inhibitors that have greater selectivity than previous JAK inhibitors, thereby increasing efficacy while minimizing side effects. Upadacitinib is indicated for the treatment of certain chronic inflammatory conditions, including rheumatoid arthritis, psoriatic arthritis, atopic dermatitis, and ulcerative colitis.

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Upadacitinib works by selectively targeting JAK1-mediated cytokine signaling pathways that are involved in disease progression. This mechanism of action is effective in reducing inflammation, relieving symptoms, and enhancing quality of life. Its oral bioavailability and rapid onset of action have made it an essential part of immunomodulatory therapy.[4]

Upadacitinib is widely used in the management of immune-mediated inflammatory conditions. Some of the conditions that upadacitinib is used to treat include moderate to severe rheumatoid arthritis, psoriatic arthritis, atopic dermatitis, and ulcerative colitis.[5] Upadacitinib acts by selectively targeting Janus kinase 1 (JAK1), which is responsible for cytokine signaling. This is significant in that it enables the management of symptoms, control of disease, and generally improves outcomes in patients. Therefore, upadacitinib is an essential part of immunotherapy in modern medicine.[6]

Despite its effectiveness in the management of immune-mediated conditions, upadacitinib is associated with several side effects. Some of the side effects include upper respiratory tract infections, nausea, headaches, acne, and mild elevations in liver enzymes. However, other more serious side effects include severe infections, including tuberculosis. Other serious side effects include cardiovascular events, thrombosis, and malignancies. Malignancies include lymphomas and skin cancers. Laboratory abnormalities include elevated cholesterol levels, low white blood cell count, and anemia. Therefore, monitoring is essential in ensuring that patients using upadacitinib achieve effective outcomes.[7]

Upadacitinib molecular weight is 380.375 g/mol, molecular formula is $C_{17}H_{19}F_3N_6O$, IUPAC name is *(3S,4R)-3-ethyl-4-(3H-imidazo[2-a]pyrrolo[3-e]pyrazin-8-yl)-N-(2,2,2-trifluoroethyl)pyrrolidine-1-carboxamide*, The molecular structure of Upadacitinib was presented in Figure 1. Drug class is Janus kinase (JAK) inhibitor, and Routes of administration is Oral (by mouth). Previous literature has been noted that QbD approaches Enantiomeric Quantification[8], RP-HPLC[9], RP-HPLC combination[10], UPLC-MS/MS in plasma [11-12], UPLC[13], LC-MS/MS in plasma[14-17], biological samples using two different electrodes[18], and clinical trial[19] to developing analytical methods for Upadacitinib. The preliminary objective of this study was to develop and validate a reliable analytical method for the estimation of Upadacitinib in its pharmaceutical dosage form, along with stability assessment through formed degradation studies. To evaluate the eco-friendly nature of the developed method by applying suitable green analytical chemistry assessment tools. The proposed method will be systematically validated in accordance with recognized regulatory guidelines to ensure its suitability for routine analysis.

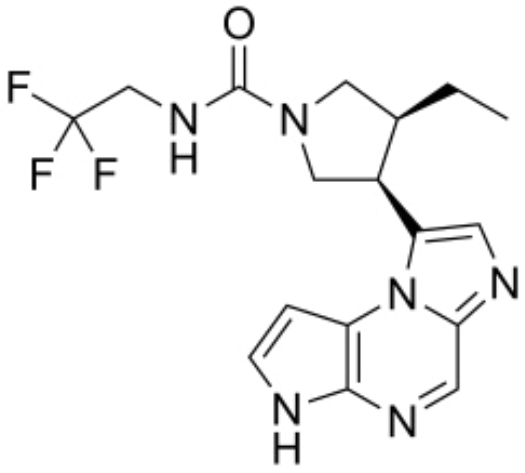

Figure 1: Chemical structure of Upadacitinib

Materials and Methods

Upadacitinib API with 99.09% purity and its Tablet formulation (Rinvoq® 30 mg) were procured from Honour labs, Hyderabad. HPLC-grade solvents includes methanol, acetonitrile, and water, In addition with AR grade chemicals includes buffer solutions, hydrogen peroxide (H_2O_2), sodium hydroxide (NaOH) and hydrochloric acid (HCl) were supplied by Merck Specialties Pvt. Ltd., Mumbai, India.

Instrumentation

The study was performed using an LC-7000 HPLC system (PEAK, India) fitted with an LC-P7000 AT VP pump. The samples introduced manually through Rheodyne injector with 20 µL fixed loop, using Hamilton syringe (USA). Detection was carried through UV detector, and data were processed using Autochro-3000 Chromatostation software (version 3.55, Zhejiang University, PRC). The other instruments used include Denver SI-234 analytical balance (Bohemia), UV-Visible spectrophotometer (Teccomp UV-2301, India), and pH meter (Systronics, India). The sample preparation was performed through GT Sonic ultrasonic bath (India) along with borosilicate vacuum filtration setup. All solutions were filtered through 0.2 µm membrane filters before analysis.

Standard solution preparation

A stock solution of Upadacitinib was prepared by accurately weigh 10 mg of drug and transfers it into a 10 mL volumetric flask. The compound was dissolved in methanol, and the volume was adjusted to the mark with same solvent to obtain 1 mg/mL (1000 µg/mL) concentration. This stock solution was further diluted with diluent to prepare working standard solutions within 30-105 µg/mL concentration range and was stored at 4°C until analysis.

Formulation solution preparation

A sample solution of formulation was prepared using Upadoz® brand containing 45 mg of Upadacitinib. An amount of tablet powder equivalent to 10 mg of drug was accurately weigh and transfer into 10 mL volumetric flask with 5 mL of methanol. The mixture was sonicated for 5 min to achieve complete dissolution. The resultant solution was filter through 0.45 µm membrane filter to remove insoluble

materials. The volume was then made up to mark with methanol and further dilutions were performed with same solvent as required for analysis.

HPLC Method Development

The development of method was initiated with selection of an appropriate detection wavelength using UV-Visible spectrophotometer. In this, standard solution (60 µg/mL) of Upadacitinib was scanned over 400-200 nm to obtain its absorption spectrum. Considering the polar nature of the drug, separation was performed on reversed-phase C18 column. Different chromatographic conditions were systematically evaluated with the variation of composition of mobile phase. These include combinations of methanol, acetonitrile, and water in different ratios with maintenance of constant flow rate of 0.5 mL/min. Multiple trials were performed using under isocratic conditions to achieve suitable separation characteristics. These experiments were conducted to identify appropriate chromatographic conditions for reliable analysis of Upadacitinib.

Method validation

The ICH guidelines[19–22] were adopted for method validation to confirm its suitability for quantitative analysis. The method specificity was evaluated with the analysis of blank, standard, and formulation samples to ensure that no interference possible at retention time of Upadacitinib. The chromatograms obtained from different preparations were carefully correlates to verify the ability of method to selectively identify analyte in presence of excipients. Retention time consistency further confirms the reliable identification capability of proposed method. The system suitability was assessed by six repeated injections of standard solution, and parameters such as peak area, tailing factor, and theoretical plate count were examined. The acceptance criteria includes %RSD below 2%, tailing factor not exceeds 2, and theoretical plates greater than 2000 confirms adequate system performance.[23]

Method sensitivity was established with the determination of detection limit (LOD) and quantification limit (LOQ). These concentrations represent the lowest detectable and measurable concentrations of method. These values were obtained based on signal-to-noise ratios of 3:1 and 10:1, respectively, using progressively diluted solutions.

Method linearity was assessed with the preparation and analysis of a series of standard solutions from LOQ level up to higher concentrations using a stock solution (1 mg/mL). Each level was injected in triplicate (20 µL), and calibration curve was constructed by plotting peak area against concentration. Regression analysis confirms relationship between concentration and response. Precision was evaluated at concentration of 60 µg/mL by performing six replicate analyses. Both repeatability (intra-day) and intermediate precision (inter-day over three days) were tested, and results were expressed as %RSD. Accuracy was determined through recovery studies using standard addition technique at 50%, 100%, and 150% levels, where known amounts of Upadacitinib were added to placebo and analyzed.

The ruggedness was verified by two different analysts under similar experimental conditions, and consistency of results was expressed as %RSD. Robustness was examined with the introduction of small deliberate changes in chromatographic conditions includes mobile phase composition, flow rate, and detection wavelength (±5%), and their impact on method performance was assessed. Finally, the method was applied for the analysis of market tablet formulations. The samples prepared were analyzed under optimized conditions, and assay results were calculated using calibration curve proves the applicability of method for routine quality control analysis. [24]

Stress degradation study

The stress degradation studies were conducted to evaluate stability of drug under different stress conditions over 24 hours. In acid degradation, 50 mg of drug was treated with 0.1 N HCl, follows neutralization and dilution to 60 µg/mL before analysis. Base degradation was performed in similar manner using 0.1 N NaOH. The oxidative stress was induced with the treatment of drug with 3% hydrogen peroxide. Then, the solution was neutralized and diluted to the required concentration. In photolytic studies, drug sample was exposes to UV light at 247 nm for 24 hours, while thermal degradation was assessed with expose of sample to thermal temperature at 60°C for the same duration. After each stress condition, samples were appropriately prepared and analyzed in the proposed method. These studies help to understand the stability behavior of drug under different environmental and chemical conditions that ensure its quality and performance during storage.[25]

Assessment of method greenness

The greenness of the developed RP-HPLC method for the estimation of Upadacitinib was assessed using two well-established tools, namely the Analytical Greenness (AGREE) metric and the Green Analytical Procedure Index (GAPI). These tools were employed to comprehensively evaluate the environmental sustainability and eco-friendliness of the proposed analytical method in accordance with the principles of Green Analytical Chemistry. Both tools examine different aspects of method development—from sample preparation to waste disposal—thus providing a holistic understanding of the method's environmental impact.

In the AGREE evaluation, the assessment was carried out using the AGREE software (online calculator) developed by Pena-Pereira and co-workers, which quantifies the adherence of the analytical procedure to the twelve principles of Green Analytical Chemistry. The method-related parameters, including the nature and volume of solvents, energy consumption, sample size, automation, waste generation, and safety of reagents, were systematically entered into the software interface. Each principle was scored automatically

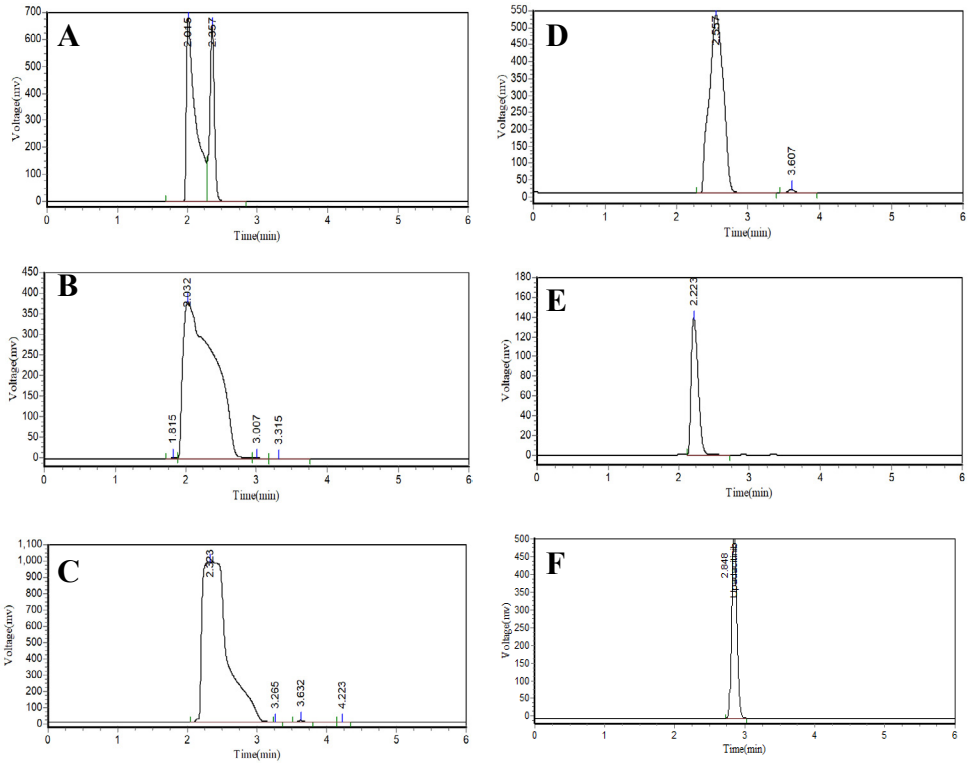

Figure 2: Chromatograms obtained for Upadacitinib under different experimental conditions

based on the provided data, and default weightings were used to maintain uniformity. The AGREE tool generated a circular, clock-like pictogram displaying individual scores for each principle, along with a final cumulative greenness score ranging from 0 (least green) to 1 (most green). The visual and numerical outputs were saved for subsequent documentation and interpretation. [26]

The GAPI approach was simultaneously employed to provide a visual evaluation of the method's environmental performance throughout all analytical stages. The GAPI assessment, fifteen parameters that covers the entire workflow such as sample collection, preparation, reagent selection, instrumentation, and waste treatment were analyzed. Each parameter was classified and color-coded as green (low environmental impact), yellow (moderate impact), or red (high impact), based on the inherent characteristics of the method. Environmentally benign choices such as the use of ethanol–water as mobile phase, minimal sample volume, and reduced analysis time were particularly considered favorable in the scoring process. The resulting GAPI pictogram provided a semi-quantitative visual representation of the overall greenness profile of the developed method. [27]

RESULT AND DISCUSSIONS

The method development was systematically performed to achieve optimal chromatographic conditions for reliable analysis of Upadacitinib. Initially, detection wavelength was selected to scan standard solution (60 µg/mL) using a UV-Visible spectrophotometer. The absorption spectrum exhibits maximum at 232 nm and was chosen for subsequent analysis due to its suitability for sensitive detection.

A series of chromatographic trials were conducted with the alteration in composition of mobile phase with maintenance of constant 0.5 mL/min flow rate and detection at 232 nm. In Trial 1 (Figure 2A), ethanol and 0.1% formic acid in the ratio of 75:25 (v/v), the chromatogram displays poor peak shape with splitting, although the baseline remains stable, indicates partial separation capability. Trial 2 (Figure 2B) was performed with ethanol and 0.1% formic acid ratio to 25:75 (v/v), results in a broad and asymmetric peak along with baseline disturbances. This chromatographic response makes the condition unsuitable. Then, Trial 3 (Figure 2C) was employed with ethanol and 0.1% formic acid in 50:50 (v/v). This condition produces wide peak with excessive tailing and unstable baseline with system suitability parameters falls outside acceptable limits.

Further modification in Trial 4 (Figure 2D), using ethanol and 10 mM ammonium acetate in an 80:20 (v/v), produces broad peak with significant tailing with baseline free from interference. In Trial 5 (Figure 2E), using mobile phase ratio to ethanol and 10 mM ammonium acetate (75:25, v/v) improves peak sharpness. However, peak symmetry and response were still not satisfactory in this condition. Finally, Trial 6 (Figure 2F), with ethanol and 10 mM ammonium acetate in a 60:40 (v/v) under isocratic conditions, provides well-defined, sharp, and

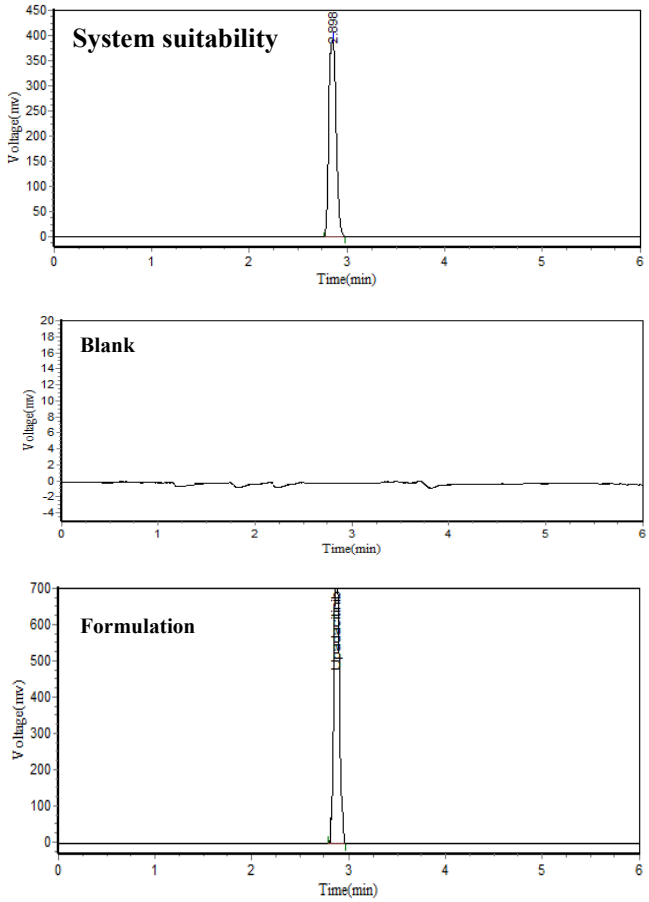

Figure 3: Chromatogram observed for Upadacitinib

symmetrical peak within 6 min runtime at system pressure of 6.2 ± 3 MPa. These optimized conditions demonstrate acceptable system suitability and were therefore selected for further validation of the method.

Chromatograms observed in Trail 1 to 5 were given in figure A to E respectively whereas optimized chromatogram in F.

Method Validation

The method specificity was proved by comparison of chromatograms observed from blank, standard, and formulation samples. The chromatographic results show no interfering peaks at retention time of Upadacitinib. These is no response was detected in the blank solution indicates the absence of matrix interference. The retention time remains consistent at 2.8 min across all injections, demonstrates reliable identification of the analyte and good selectivity of method. In the optimized chromatographic conditions, the system suitability parameters were noticed to be within acceptable limits. The peak display good symmetry with tailing factor of 1.03, and column efficiency of 5,813 theoretical plates. These results confirm that the method provides adequate performance for analysis. The corresponding chromatograms are presented in Figure 3.

Table 1: Linearity results for the Upadacitinib

S. No	*Concentration in µg/mL*	*Area response*
1	30	390363.7
2	45	566339.3
3	60	753284.2
4	75	924832.1
5	90	1098221.7
6	105	1278936.8

The method sensitivity was evaluated with the assessment of LOD and LOQ, which were determined to be 0.025 µg/mL and 0.082 µg/mL, respectively. These low values indicate that the method has the capability to detect and accurately measure very small amounts of Upadacitinib, confirms its high sensitivity. The method linearity was assessed with the preparation of a series of standard solutions with courage of wide concentration range includes working concentration of 60 µg/mL. The mean peak areas obtained after analysis of these solutions were used to construct a calibration against concentration. The method displays strong linear relationship over 30-105 µg/mL concentration range for Upadacitinib. The regression equation obtained was y = 11829x + 36893, with a correlation coefficient (R^2) of 0.9999, indicates excellent linearity and proportional response within the studied range. The linearity data was presented in Table 1 and its corresponding calibration curve was presented in Figure 4.

The method precision was evaluated in terms of repeatability (intra-day) and intermediate precision (inter-day). The %RSD values were observed as 0.36% for intra-day and 0.42% for inter-day analysis. Both these %RSD values were well within acceptable limit of 2%. These results indicate that this method provides consistent and reliable measurements for Upadacitinib. The detailed precision data are presented in Table 2.

Ruggedness was assessed by performance of analysis under similar conditions by two different analysts on different days using same instrument. A standard solution of 60

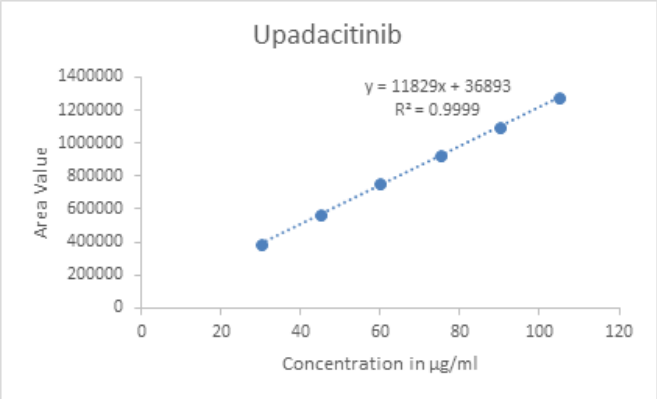

Figure 4: Linearity calibration curve observed for Upadacitinib in the proposed method

Table 2: Results observed in the evaluation of Precision and Ruggedness test for the proposed method

S. No	*Intraday Precision*	*Interday Precision*	*Ruggedness*
1	757584.2	750394.6	758364.7
2	752397.9	754732.7	759203.1
3	758092.6	758433.2	752876.2
4	758498.1	752339.8	757925.3
5	753027.3	754789.2	759725.1
6	754087.5	758303.8	752186.2
% RSD	0.36	0.42	0.43

µg/mL was analyzed in six replicates by each analyst. The %RSD values were noticed to be 0.44%, which was within permissible limit of less than 2%. This demonstrates that the method was reproducible and not significantly affects with the variations in analyst proves its robustness in routine analysis (Table 2).

Method robustness was evaluated with the introduction of small deliberate changes in analytical conditions like slight variations in detection wavelength, buffer pH, and mobile phase composition. The standard solution of Upadacitinib (60 µg/mL) was analyzed under these modified conditions. The observed percentage variation was within 0.05% to 0.86% range and values well within desired limit of less than 2%. These results indicate that minor changes in experimental parameters do not significantly affect the analytical performance confirms that the method was reliable and stable under varied conditions. The robustness data are summarized in Table 3.

Method accuracy was evaluated through recovery studies by spiking pre-analyzed sample solutions with known amounts of Upadacitinib at 50%, 100%, and 150% of the label claim. The %RSD values were 0.56%, 0.27%, and 0.35%, respectively and these values were within acceptable limit of less than 2%. The % recoveries were noticed to be 99.38% to 100.47% range, indicate that method provides accurate and reliable results without interference from excipients. These findings confirm method suitability for precise quantification of Upadacitinib in pharmaceutical formulations. The detailed recovery data was presented in Table 4.

The proposed method was applied to assess content of Upadacitinib in Upadoz® tablet formulation. The assay result was observed to be 99.12% (59.47 µg/mL) and was noticed to be close to the labeled amount. The method consistently produces an assay values above 98%, indicates good accuracy and reliability. These results demonstrate that method was suitable for routine quality control analysis of Upadacitinib in both bulk drug and finished dosage forms.

The HPLC analysis of Upadacitinib under various stress conditions displays no interference between drug peak and its degradation products. The peak purity results remain within acceptable limits in all cases, confirms that Upadacitinib peak was homogeneous and well resolved. These observations demonstrate the method specificity and its ability to act as a stability-indicating assay. The method successfully enables the separation, identification, and quantification of Upadacitinib under different stress conditions, includes acidic, basic, oxidative (peroxide), thermal, and photolytic degradation. This confirms that method was suitable for stability studies and reliable evaluation of degradation behavior. The number of degradation products formed under different conditions was determined as 2, 0, 3, 0 and 0 under acidic, basic, peroxide, thermal, and UV light stress conditions, respectively. Among these, maximum % degradation was observed under peroxide stress conditions; i.e., under peroxide stress, % degradation was 8.76% with the formation of three degradation products. On the other hand, minimum % degradation was observed under base, thermal and UV stress conditions, determined as 3.81, 5.07 and 2.12, respectively, showing higher stability of the drug substance under these conditions. All the degradation products were well separated without any interference with the peak of the standard drug substance, thus it was confirmed that the method developed was stability-indicating. The comprehensive stress study chromatograms were presented in Figures 5.

Assessment of method greenness:

The greenness of the developed RP-HPLC method for the estimation of Upadacitinib was quantitatively evaluated using the Analytical GREEnness (AGREE) metric tool. The AGREE evaluation is based on the twelve principles of Green Analytical Chemistry (GAC), each represented as a segment in

Table 3: Robustness results for the Upadacitinib

S. No	*Condition*	*Change*	*Area*	*% Change*
1	Standard	No change	753284.2	--
2	MP 1	Ethanol and 10 mM ammonium acetate in 65:35 (v/v)	752829.5	0.06
3	MP 2	Ethanol and 10 mM ammonium acetate in 55:45 (v/v)	752749.1	0.07
4	WL 1	229 nm	752875.8	0.05
5	WL 2	236nm	759725.9	0.86
6	pH 1	4.2	757439.6	0.55
7	pH 2	4.4	751761.3	0.20

Table 4: Accuracy results for the reported Upadacitinib

% Recovery	*Concentration in µg/ml*			*Amount Found*	*% Recovery*	*% RSD*
	Target	*Spiked*	*Total*			
50%	30	15	45	44.72	99.38	0.56
	30	15	45	45.08	100.18	
	30	15	45	44.60	99.11	
100%	30	30	60	60.24	100.41	0.27
	30	30	60	60.28	100.47	
	30	30	60	59.98	99.98	
150%	30	45	75	75.21	100.29	0.35
	30	45	75	74.73	99.64	
	30	45	75	75.13	100.18	

the circular pictogram (Figure 6), with colors ranging from red (poor) to green (excellent) depending on the environmental friendliness of the analytical process. The central numerical value represents the overall greenness score on a scale from 0 (least green) to 1 (most green).

The AGREE score was 0.82 was observed indicating a high level of greenness and excellent compliance with most principles of Green Analytical Chemistry. The circular diagram predominantly exhibited dark green and light green shades, signifying that most aspects of the method align well with sustainable analytical practices. The high score demonstrates that the method effectively minimizes environmental impact while maintaining analytical robustness and reliability.

The use of ethanol as the organic modifier instead of traditional toxic solvents such as acetonitrile or methanol contributed significantly to the favorable score, as ethanol

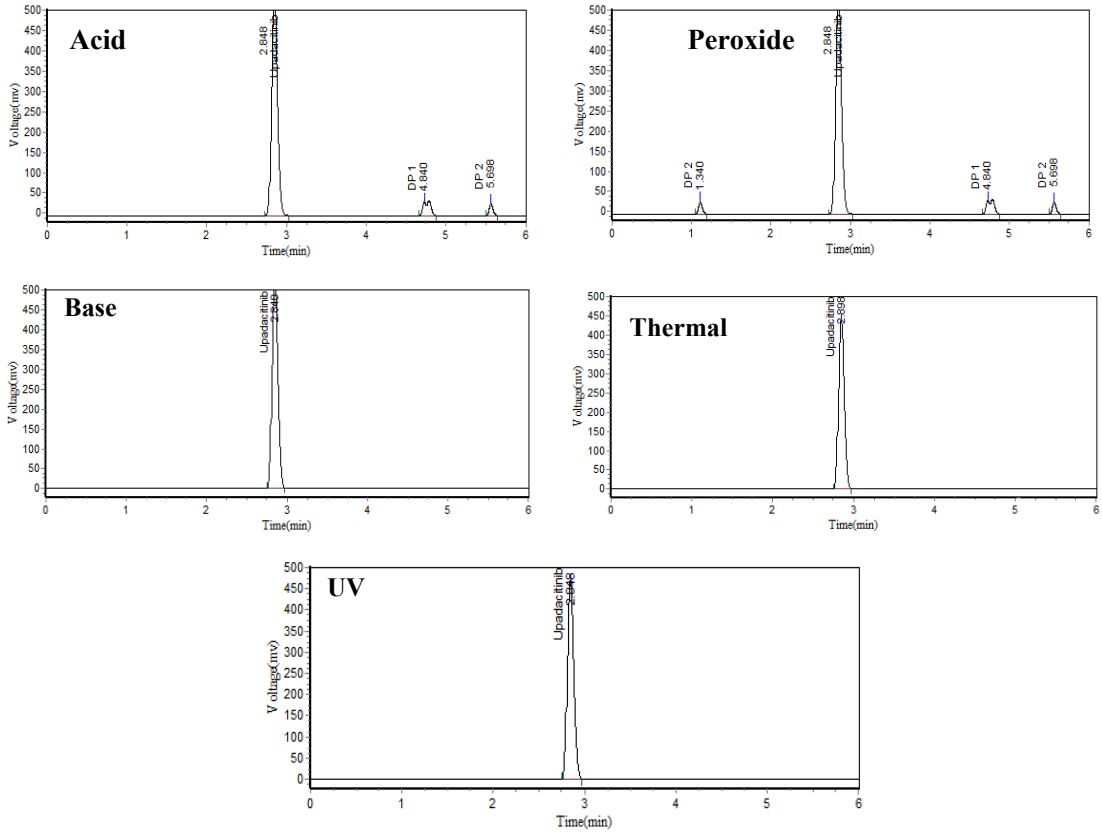

Figure 5: Stress degradation chromatograms observed in the proposed method for the analysis of Upadacitinib

Figure 6: AGREE results observed for method proposed for the analysis of upadacitinib

is biodegradable, renewable, and poses lower toxicity and disposal concerns. The mobile phase composition of ethanol and aqueous ammonium acetate buffer ensured reduced solvent consumption and lower waste generation, fulfilling principles related to safer solvent use, waste prevention, and energy efficiency. The short analysis time (approximately 8 minutes) and the moderate flow rate (0.5 mL/min) further enhanced the method's sustainability by minimizing solvent usage per run and reducing overall energy demand.

Principles associated with automation, miniaturization, and real-time monitoring achieved satisfactory ratings, as the RP-HPLC method utilizes automated sample injection and UV detection, ensuring high precision with reduced human exposure to reagents. The low sample volume and minimal reagent requirement also reflected strong compliance with

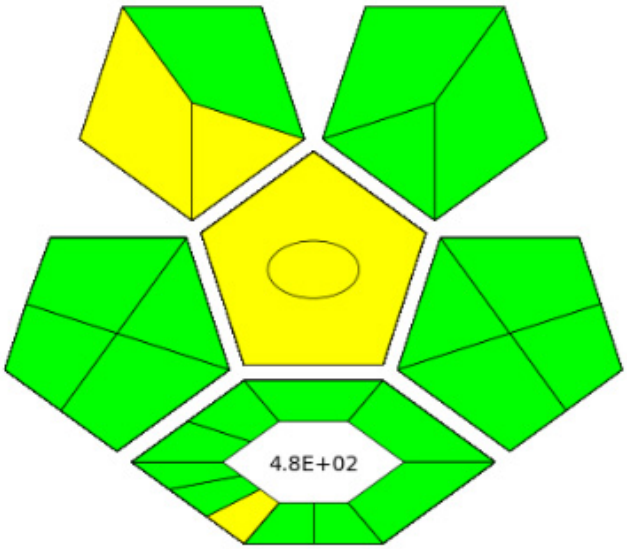

Figure 7: GAPI results observed for method proposed for the analysis of upadacitinib

green analytical principles focused on resource efficiency and operator safety. Minor limitations were noted for principles related to reagent preparation and waste treatment, as ethanol remains a flammable solvent, and small volumes of acidic or buffered waste still require proper disposal. These factors slightly influenced the scoring of principles 8 and 11, which appeared in lighter yellow-green shades.

Overall, the AGREE pictogram (Figure 6) and score of 0.82 confirm that the developed RP-HPLC method is environmentally sustainable, operator-safe, and efficient, meeting the core objectives of Green Analytical Chemistry. The results validate that this method not only achieves accurate and precise quantification of Upadacitinib but also demonstrates a strong commitment to eco-friendly analytical practices, making it suitable for routine quality control and stability studies with minimal ecological footprint.

The greenness profile of the proposed RP-HPLC method for the estimation of Upadacitinib was comprehensively evaluated using the Green Analytical Procedure Index (GAPI) tool. The GAPI approach provides a holistic evaluation of the environmental impact of the analytical procedure, covering all stages of the workflow—from sample collection and preparation to instrumental analysis and waste management. The obtained GAPI pictogram, as shown Figure 7, illustrates the method's greenness through a five-segment pentagonal pattern, where each segment represents a different stage of the analytical process. The color code—green (low environmental impact), yellow (moderate impact), and red (high impact)—visually indicates the sustainability level of each step.

In the present study, the GAPI pictogram revealed a predominance of green-colored zones, confirming that most analytical steps were environmentally benign and complied well with the principles of Green Analytical Chemistry. The central pentagon, representing the general method type and its overall environmental impact, appeared yellow, indicating a moderately sustainable analytical approach. This moderate rating is attributed to the inherent use of solvents in liquid chromatography and the requirement for instrument power, both of which cannot be completely eliminated in HPLC-based methods. Nevertheless, the absence of any red zones signifies that no step in the procedure poses a significant environmental hazard.

The segments corresponding to sample collection and preparation were entirely green, reflecting minimal reagent use, simple dissolution and filtration steps, and the absence of toxic derivatizing or extraction agents. The method employs ethanol–water and ammonium acetate buffer as the mobile phase, both of which are safe, biodegradable, and non-toxic, thereby eliminating the hazards associated with conventional solvents like acetonitrile or methanol. The instrumental analysis section also displayed a green coloration, demonstrating the method's efficiency in terms of solvent volume (only 4–5 mL per run) and energy consumption (low flow rate and short runtime of about 8 minutes).

The waste generation and treatment segment showed a mixture of green and yellow zones, indicating that while the total solvent waste is relatively small and composed of non-hazardous materials, ethanol remains a flammable solvent and thus requires appropriate handling and disposal. The numerical value (4.8E+02) displayed in the center corresponds to the E-factor of 475, meaning that approximately 475 g of waste is generated per gram of analyte analyzed. Despite this numerical value, which is typical for HPLC-based analyses, the low-toxicity nature of the waste renders the method significantly greener than conventional chromatographic procedures.

Overall, the GAPI results confirm that the developed RP-HPLC method for Upadacitinib demonstrates excellent environmental compatibility, with the majority of its analytical steps classified as green and only minor aspects (related to solvent flammability and instrument energy use) showing moderate impact. The combined evaluation indicates that the method is sustainable, safe, and environmentally responsible, making it highly suitable for routine quality control applications and stability studies within a green analytical chemistry framework.

A review of the literature indicated that previously reported QbD-based analytical methods for Upadacitinib showed linearity over a relatively narrow concentration range. In contrast, the present method demonstrated a significantly wider linearity range of 30-105 µg/mL, enabling accurate quantification across low to high concentration levels. Furthermore, the sensitivity of the developed method was confirmed by low detection and quantification limits, with LOD and LOQ found to be 0.025 µg/mL and 0.082 µg/mL, respectively, for Upadacitinib. The broader linear range combined with improved sensitivity highlights the capability of the proposed method for comprehensive analysis. Based on the comparative literature survey, no reported method was found to cover such an extended concentration range with comparable detection limits, indicating that the developed method is novel, sensitive, and suitable for routine analytical applications.

CONCLUSION

A simple, reliable, and stability-indicating RP-HPLC method was proposed for the assessment of Upadacitinib in both bulk drug and pharmaceutical formulations. The method produces a clear and well-resolved peak at 2.8 min, without any interference from excipients or degradation products. System suitability results prove the good performance, with tailing factor of 1.03 and 5813 theoretical plates indicates efficient separation. The method displays high sensitivity, with LOD and LOQ values of 0.025 µg/mL and 0.082 µg/mL, respectively. A strong linear relationship was observed over 30-105 µg/mL (R^2 = 0.9999). The precision results were satisfactory, with %RSD values of 0.36 for intra-day and 0.42 for inter-day analysis. Accuracy was within acceptable limits, with recovery values in 99.38% to 100.47% range. The method also demonstrates good ruggedness and robustness with %RSD and % variation of below 2%. Forced degradation studies confirm that method can effectively detect changes in drug under different stress conditions and proves its stability-indicating nature.

Besides the assessment of the method's performance, the environmental impact of the method was evaluated using the AGREE and GAPI tools. The high value of the AGREE tool (0.82) shows the strong compliance of the method with the principles of green analytical chemistry. Moreover, the use of ethanol as a non-toxic solvent and the ammonium acetate buffer minimized the toxicity risk of the method. In addition, the decrease in the run time and the flow rate minimized the solvent consumption and the generation of wastes. Moreover, the results of the GAPI tool showed the eco-friendly character of the method, with a predominance of the green areas of the tool and the absence of critical environmental concerns. Moreover, the value of the E-factor (475) of the method is acceptable due to the low toxicity and biodegradability of the solvents used in the method and the fact that the method is based on the HPLC technique. Overall, the method complies with ICH guidelines and was suitable for routine quality control, assay, and stability studies of Upadacitinib. It was successfully applied to the analysis of formulation tablets and proves its practical applicability.

ACKNOWLEDGEMENT

The Management, Principal and staff of the Victoria College of Pharmacy, India are gratefully acknowledged by the authors for the facilities provided and ongoing support for the completion of the current study.

CONFLICT OF INTEREST

Nil

REFERENCES:

1. Swarbrick J, Boylan JC. Encyclopedia of pharmaceutical technology. New York. 1988; 1:217-24.
2. Siva Prasad S, Venkateswara Rao A, Bikshal Babu K. Quality by design (QBD) based development and validation of an HPLC method for the estimation of lurasidone in pharmaceutical formulations. Journal of Critical Review. 2020;7(18):2155-2161.
3. Bikshal Babu K, Madhu Babu K. Caffeine in Chocolates a Hazardous Sign to Children's Health. International Journal of Pharmaceutical & Biological Archives. 2011;2(6):1757-1760.
4. Mohamed MF, Zeng J, Marroum PJ, Song IH, Othman AA. Pharmacokinetics of Upadacitinib with the Clinical Regimens of the Extended-Release Formulation Utilized in Rheumatoid Arthritis Phase 3 Trials. Clinical Pharmacology in Drug Development. 2019; 8(2):208-216.
5. Biggioggero M, Becciolini A, Crotti C, Agape E, Favalli EG. Upadacitinib and filgotinib: the role of JAK1 selective inhibition in the treatment of rheumatoid arthritis. Drugs Context. 2019;8:212595.
6. Schwartz DM, Kanno Y, Villarino A, Ward M, Gadina M, O'Shea JJ. JAK inhibition as a therapeutic strategy for immune and

inflammatory diseases. Nature Reviews Drug Discovery. 2017;17(1):78.
7. Madhu Babu K, Bikshal Babu K. A review on brazilian pepper plant: *Schinus molle*. Journal of Atoms and Molecules (Jamonline). 2012;2(2):6–13.
8. Kanaan BM, Algohary AM, Alhalafi MH, Ibrahim AM. Analytical Quality by Design for Chiral Pharmaceuticals: A Robust HPLC Method for Upadacitinib Enantiomeric Quantification. Chirality. 2025;37(5):e70036.
9. Kashid AM, Kaldate DP. Development and validation of a green stability-indicating RP-HPLC method for upadacitinib: a selective Janus kinase (JAK) inhibitor. Discover Chemistry. 2025;2(1):218.
10. Ibrahim Baje S, Madhavi N. Related Substances Method Development and Validation of Axitinib, Zanubrutinib and Upadacitinib Using RP-HPLC and its Degradation Products were Characterized by Using LC-MS/MS. International Journal of Pharmacology Investigation. 2023;13(1):113-121.
11. Li J, Chen C, Wang J, Ye Z, Pan L, Liu Z, Tang C. Simultaneous measurement of upadacitinib and methotrexate by UPLC-MS/MS and its pharmacokinetic application in rats. Journal of Chromatography B. 2022;1188:123071.
12. Wang MJ, Zhao YH, Fan C, Wang YJ, Wang XQ, Qiu XJ. Development of an UPLC-MS/MS method for the quantitative analysis of upadacitinib in beagle dog plasma and pharmacokinetics study. Drug Design. Development and Therapy. 2021;4167-4175.
13. Rajya Lakshmi N, Gummadi S. A UPLC method development and validation study of Upadacitinib and its impurities in extended–release oral tablet dosage forms. In Annales Pharmaceutiques Françaises. 2024;82(5):780-791.
14. Venkateswara Y, Jithendra C. Quantification of an anti-rheumatic agent: Upadacitinib in biological fluid (plasma) by LC-MS/MS. journal of pharmaceutical negative results. 2022; 13(6):3279-3295.
15. Martens-Lobenhoffer J, Tomaras S, Feist E, Bode-Boeger SM. Quantification of the janus kinase 1 inhibitor upadacitinib in human plasma by LC-MS/MS. Journal of Chromatography B. 2022;1188:123076.
16. Wu JJ, Lin L, Yan JJ, Kong JH, Xuan QL, Gao X. Development and validation of a sensitive LC-MS/MS assay for determination of upadacitinib in human plasma and its application in patients with inflammatory bowel disease. Journal of Pharmacological and Toxicological Methods. 2025;131:107581.
17. SubhashinI K, Raju RR, Gorumutchu GP. A liquid chromatographic method for the reliable quantification of Upadacitinib and its specified impurities. International Journal of Applied Pharmacology. 2025;17(4):420-34.
18. Cetinkaya F, Kaya A, Bellur Atici E, Ozkan SA. Development of a sensitive and simple method for the analysis of upadacitinib in biological samples using two different electrodes. Ionics. 2025;31(1):1003-1017.
19. Hussain M, Aallam A, Hetta H, Hamed A. OP0088 Upadacitinib as a Potential Therapeutic Option in Treatment of Scleroderma related ILD: Randomized double blind clinical trial. Annals of the Rheumatic Diseases. 2025;84:77.
20. ICH guideline Q14 on analytical procedure development, 2022.
21. ICH guideline, stability testing of new drug substances and products Q1A(R2). 2003.
22. ICH Topic Q 2 (R1), Validation of Analytical Procedures: Text and Methodology, 2006.
23. Bikshal Babu K, Madhu Babu K. New spectrophotometric methods for the quantitative estimation of Refaximin in formulations. Journal of Atoms and Molecules. 2011;1(1):48.
24. Rajesh Varma Bhupatiraju, Bikshal Babu Kasimala, Lavanya N, Fathima Sayed. Structural evaluation of degradation products of Loteprednol using LC-MS/MS: Development of an HPLC method for analyzing process-related impurities of Loteprednol. Analytical science technology. 2024;37 (2):98-113.
25. ICH Topic Q 2 (R2), Validation of Analytical Procedures: Text and Methodology, 2022.
26. Rajesh Bhupatiraju V, Pavani P, Subhashini E, Rekha K, Bikshal Babu Kasimala. Green Analytical Approach for HPLC Method Development for Quantification of Sorafenib and Its Pharmacopeia Impurities: LC–MS/MS Characterization and Toxicity Prediction of Stress Degradation Products. Separation science plus. 2024; 7(9):e202400106. Available from: doi.org/10.1002/sscp.202400106
27. Ravinder B, Hemant Kumar T, Vijay Srinivas P, Likhitha A, Sambasiva Rao T, Naveena G. Analytical quality by design approach in RP-HPLC method development for the quantification of mirabegron and solifenacin succinate in pharmaceutical formulation. Letters in applied NanoBioScience. 2024;14(1):1-12.

How to cite this article: Prudhvi SN, Gunda RK, Ch LS, Rani R, Akhila T, Meghana K, Sharon P, Kavya VSS. Development, Validation, and Forced Degradation Evaluation of a Green Stability-indicating RP-HPLC method for Upadacitinib in Bulk and Tablet Dosage Forms . Journal of Applied Pharmaceutical Sciences and Research. 2026; 9(2):56-65 Doi: 10.31069/japsr.v9i2.05