ISSN: 2231-3656

International Journal of Pharmacy and Industrial Research (IJPIR)

IJPIR |Vol. 16 | Issue 4 | Oct – Dec 2026

www.ijpir.com

DOI: https://doi.org/10.61096/ijpir.v16.iss4.2026.1083-1091

Development and Validation of Spectrophotometric Method for the Estimation of Upadaticinib in Bulk and Pharmaceutical Dosage Form

[1]Mulukuntla Lahari, [1]Malothu Ramya Sri, [1]Sriram Lahari, [1]Rafya Sultana, [1]Kummari Akhil Varun, *[2]Neelima kudumula, [3]Duddagi Suchitra

[1]Research students, Department of Pharmaceutical Analysis, Sarojini Naidu Vanita Pharmacy Mahavidyalaya (Co-Ed) Affiliated to Osmania University, Tarnaka, Hyderabad, Telangana, India.

*[*2]Professor, Department of Pharmaceutical Analysis, Sarojini Naidu Vanita Pharmacy Mahavidyalaya (Co-Ed), Affiliated to Osmania University, Tarnaka, Hyderabad, Telangana, India.*

[3]Associate Professor, Department of Pharmaceutical Analysis, Sarojini Naidu Vanita Pharmacy Mahavidyalaya (Co-Ed), Affiliated to Osmania University, Tarnaka, Hyderabad, Telangana, India.

Corresponding Author: ***Neelima kudumula**
E-Mail ID: Neelima_kudumula@yahoo.com

Published on:

13.11.2026

Published by:

Futuristic Publications

2026| All rights reserved.

Creative Commons Attribution 4.0 International License.

Abstract: Upadacitinib is a selective Janus kinase (JAK1) inhibitor used in the management of rheumatoid arthritis and other inflammatory conditions. The present study aims to develop and validate a simple, precise, and economical UV spectroscopic method for the quantitative estimation of Upadacitinib in bulk and pharmaceutical dosage forms. Upadacitinib exhibited maximum absorbance (λmax) at 233nm when scanned in the UV region using methanol as the solvent.

The method obeyed Beer-Lambert's law in the concentration range of 2–10 µg/mL, with a correlation coefficient (R^2 = 0.9991), indicating excellent linearity. The developed method was validated in accordance with International Council for Harmonisation guidelines. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.39 µg/mL and 1.17 µg/mL, respectively, demonstrating good sensitivity.

The precision of the method was confirmed with %RSD values of less than 2% for both intra-day and inter-day studies. The accuracy, determined by recovery studies at 50%, 100%, and 150% levels, ranged between 98.5% and 101.2%, indicating the reliability of the method. The method showed good reproducibility and no significant interference from excipients present in the formulation.

The proposed UV spectroscopic method is simple, sensitive, accurate, and cost-effective, making it suitable for routine quality control analysis of Upadacitinib in bulk and pharmaceutical dosage forms.

Keywords: Upadacitinib, UV spectrophotometric method, Validation.

INTRODUCTION:

Upadacitinib is an orally administered, selective Janus kinase 1 (JAK1) inhibitor that has emerged as an important therapeutic agent in the management of autoimmune and inflammatory disorders. It belongs to a newer class of targeted synthetic disease-modifying antirheumatic drugs (tsDMARDs), designed to modulate intracellular signalling pathways involved in inflammation.

The drug is primarily approved for the treatment of conditions such as rheumatoid arthritis, psoriatic arthritis, atopic dermatitis, and ulcerative colitis. Upadacitinib exerts its pharmacological effect by selectively inhibiting the JAK1 enzyme, which plays a crucial role in the signalling of pro-

inflammatory cytokines. By blocking this pathway, the drug reduces inflammation, alleviates symptoms, and helps prevent disease progression.

Compared to non-selective JAK inhibitors, Upadacitinib offers improved selectivity towards JAK1, thereby minimizing adverse effects associated with JAK2 and JAK3 inhibition. This selectivity contributes to its favourable efficacy and safety profile, making it a preferred option in patients who show inadequate response to conventional therapies such as methotrexate or biologics.

In pharmaceutical analysis, the development of simple and reliable analytical methods for the estimation of Upadacitinib is essential for quality control and regulatory compliance. Among various analytical techniques, UV spectrophotometry is widely used due to its simplicity, cost-effectiveness, and rapid analysis. Therefore, the present study focuses on the development and validation of a UV spectroscopic method for the accurate quantification of Upadacitinib in bulk and pharmaceutical dosage forms. After reviewing literature, LCMS/MS [2,5,10,12,14,18] UPLC MS/MS [1,6,8,15] RP-HPLC [3,4,17] HPLC MS/MS [13,16,19] and no UV-Visible spectrophotometric method [10, 11] has been reported till date for determination of Upadacitinib. As Upadacitinib is a recently launched antirheumatic drug, a validated UV spectrophotometric method for determining Upadacitinib in pharmaceutical preparations is needed. In this study, we developed and validated a new spectroscopy method for the evaluation of Upadacitinib in bulk and tablet dosage form according to ICH recommendations. [20-22]

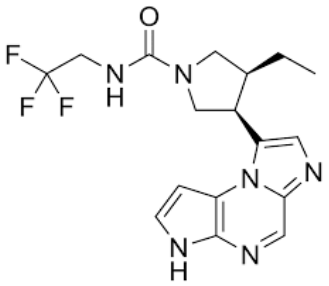


Fig 1: Structure of Upadacitinib

Table 1: Solubility study (25 °C) of Upadacitinib

Solvent	Solubility
Water (H_2O)	Insoluble
Acetone (CH_3COCH_3)	Slightly soluble
Ethyl acetate	Slightly soluble
Methanol	Completely Soluble
Acetonitrile	Completely Soluble

MATERIALS AND METHODS

Equipment:

The proposed work was carried out on a UV-1800 make is **SHIMADZU** UV- Visible spectrophotometer, with a 1 cm quartz matched cells, weighing was done on electronic balance **(Shimadzu-BL220H**), sonicated with **Sonica**, Ultrasonic cleaner, Spincotech PVT LTD.

Chemicals and reagents:

Upadacitinib standard was obtained as gift sample from MSN Laboratories, Hyderabad, **(Upadoz 15)** tablets with label claim 15 mg manufactured by MNS Pharmaceuticals were purchased from local market.

Preparation of Stock and Working Solution:

As the drugs was found to be soluble in methanol. The standard stock solution was prepared by dissolving 10 mg of Upadacitinib with little amount of water and then the volume was made upto 10 ml with methanol to acquire a concentration of 1000 µg/ml and sonicated for 10 minutes to ensure complete solubilization. A working standard solution of 10 µg/ml was prepared by appropriately diluting the stock solution with methanol

Determination of wavelength of maximum absorption:

The drugs maximum absorbance was discovered was found to be at 233 nm as shown in **Figure 2** when 10µg/ml of standard Upadacitinib was scanned in UV spectrophotometer

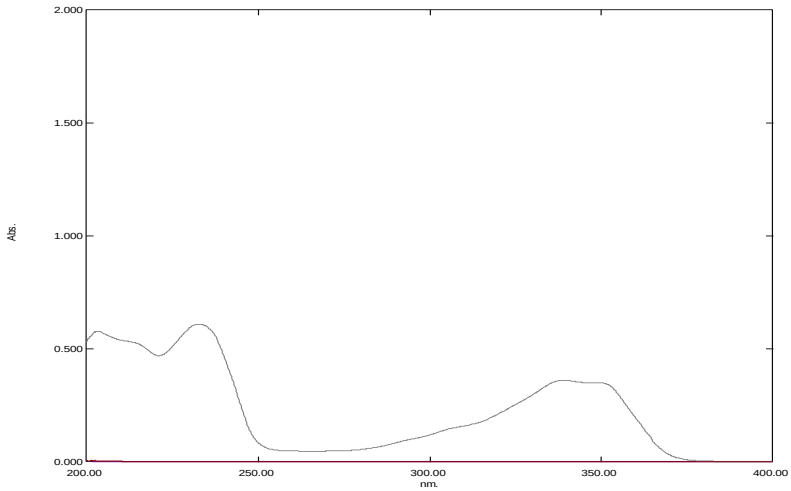


Fig 2: λmax of Upadacitinib

ANALYSIS OF THE MARKETED FORMULATION:

10 tablets were weighed and powdered. Powdered tablet equivalent to 50 mg of Upadacitinib was accurately weighed and transferred into 50 ml volumetric flask, dissolved in methanol sonicated, and finally made up the volume with methanol. The resulting solution was filtered through Whatman filter paper and from the filtrate 1ml of solution was taken in 10ml volumetric flask and made upto the mark using methanol. The volume was adjusted with methanol up to 10 ml to get 100 µg/ml solution and further diluted to prepare a concentration of 10 µg/ml. Excipients normally present in tablets did not show any interference. The results of measuring the absorption using a selected wavelength are displayed in table 1**.**

Weight of 10 Tablets (eq. to 150mg =4.9586 g

10 Tablets average weight (eq.to 15mg) = 4.9586/10 = 0.4956 g

Powder weight taken for assay (eq.to 50mg) =1.652 g

$$\text{Weight to be taken} = \frac{\text{Average weight} \times \text{Equivalent Weight}}{\text{Label claim}}$$

$$= \frac{1.652\ \text{g} \times 0.15\ \text{g}}{0.015\ \text{g}} = 16.52\ \text{mg}$$

$$\text{Assay} = 100 * \frac{\text{Absorbance Sample}}{\text{Absorbance Standard}} * \frac{\text{Concentration of Standard}}{\text{Concentration of Sample}}$$

Table 1: Percentage assay of Upadacitinib

S. No	Brand Name	Available form	Label claim(mg/tablet)	Assay (content in mg)	% Assay
1	Upadoz 15	Tablet	15mg	14.8mg	98.6%

METHOD VALIDATION

Methodology validation is the process of building narrative evidence that a system, procedure, or movement has been implemented or tested and maintained a desired level of consistency through all phases. Approved scientific strategies are essential for improving diagnostic techniques and have been time and again tested for specificity, linearity, accuracy, precision, range, limits of detection, and quantization

cutoffs. In summary, the development and approval of a systematic strategy confirm that accurate and reliable potency estimation of medicinal products has been performed.

Validation Parameters

Validation parameters are used to provide documented evidence that the performance of a method meets the requirements of the intended analytical application. The purpose of the verification is to demonstrate that the analytical results obtained using a particular method are suitable for that purpose and are as specified below.

Method Validation:

This study was conducted to develop a new inexpensive and convenient method for the spectroscopic determination of Upadacitinib. The method was tested for linearity, accuracy, precision and reliability according to the ICH guidelines.

Linearity

The Linear relationships must be evaluated using a variety of analysis methods. A minimum of 5 concentrations is recommended to establish linearity. It can be directly detected with API by diluting the standard stock solution with the proposed method. The correlation coefficient, the point of intersection with the ordinate, and the slope of the regression line should be provided.

Accuracy

To ensure the reliability of the above method, recovery studies were performed by mixing known amounts of the standard drug with formulation samples. Recovery studies were conducted at three different levels of 50%, 100% and 150% levels. The contents were analyzed using the proposed zero order, first derivative and second derivative spectroscopy**.**

Precision

The precision of the method is the degree of agreement between individual test results when the method is repeated on multiple samples of a homogeneous sample. Validation of analytical methods requires not only the performance of feature parameters, but also statistical processing of analytical data for both Intraday and Interday. These treatments determine the tolerance for variation in analytical data. The precision of analytical procedure is usually expressed as the variance, standard deviation, or coefficient of variation of a set of measurements.

Reproducibility

Reproducibility is also referred to as intra-assay precision. Reproducibility is defined as short-term accuracy under identical conditions of use.

Robustness

Robustness evaluation should be considered during the design phase and depends on the type of method being studied. The reliability of the assay with respect to intentional changes to method parameters should be demonstrated.

LOQ (Limit of Detection) and LOQ (Limit of Quantification)

Response standard deviation and determined by the linearity slope.

The detection limit (DL) can be expressed as: $DL = 3.3\ \sigma / S$

where σ = standard deviation of the response, S = slope of the calibration curve. The limit of quantification (LOQ) can be expressed as $LOQ = 10\ \sigma/S$. where σ = standard deviation of the response S = slope of the calibration curve. The slope can be estimated from the calibration curve of the analyte.

RESULTS AND DISCUSSION:

Linearity

The standard stock solution of the drug was prepared by dissolving 10 mg of the drug in 10ml of methanol and then adjusted to 10ml with water to obtain a concentration of 1000 µg/ml. Further dilution of this solution with water yields a range of solutions containing various concentrations from 2-10 µg/ml.

Absorbance was recorded at λ max 233 nm. The equation of the calibration curve using UV spectroscopy was found to be y = 0.0555x + 0.0884 for method with R^2 = 0.9993 The absorbance was plotted for each concentration to obtain a calibration curve shown in **Figure 3** and data can be found in (**Table 3).**

Calibration curve preparation

Standard stock solution was appropriately diluted to obtain final concentrations in the range of 2-10 µg/ml. For each prepared solution, absorbance was measured at the wavelengths selected above. A calibration curve was drawn between concentration and absorbance with a correlation coefficient of 0.9993 respectively. An Overlaid spectrum is shown in Figure 4.

Table 3: Standard curve data of Upadacitinib

S. No	Concentration in µg/ml	Absorbance 233nm
1	2	0.123
2	4	0.247
3	6	0.373
4	8	0.51
5	10	0.64
	Average	0.3786
	Standard deviation	0.183282
	Correlation co efficient	0.9997
	Slope	0.0649

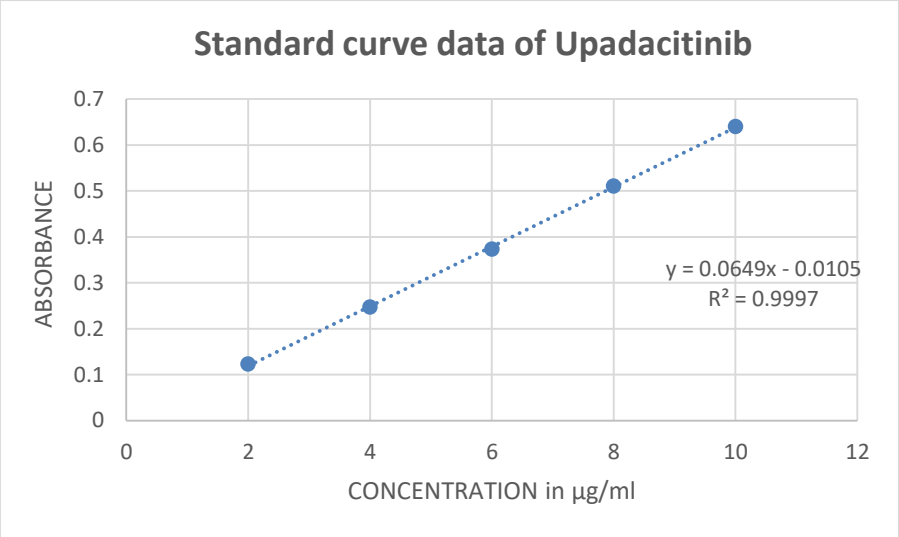


Fig 3: Linearity plot of Upadacitinib

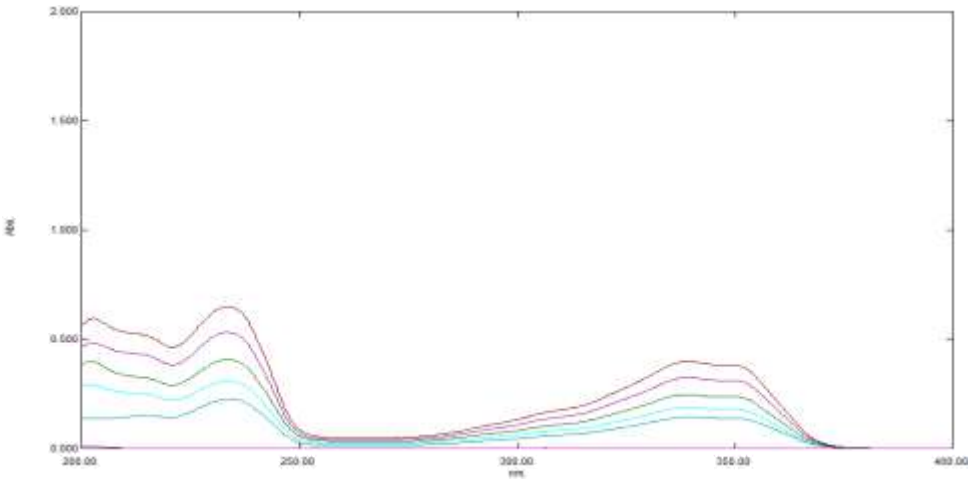


Fig 4: Overlay spectra of Upadacitinib of the concentrations used for linearity

Precision:

The intra- and inter-day precision of the proposed method was determined by analyzing the corresponding responses 6 times on the same day and on 6 different days in the same concentration (10 µg/ml) of Upadacitinib standard solution without changing the method parameters. Results are expressed as relative standard deviation (%RSD). The results obtained for intraday and inter-day variations by UV-Spectroscopy are shown in **(Table 4)**.

Table 4: Intraday and Interday precision of Upadacitinib

Sample No	Upadacitinib 233 nm	Day	Upadacitinib 233 nm
1	0.649	Day 1	0.658
2	0.645	Day 2	0.664
3	0.658	Day 3	0.661
4	0.657	Day 4	0.655
5	0.666	Day5	0.662
6	0.665		
Mean	0.664	Mean	0.66
SD	0.012	SD	0.035
%RSD	1.91	%RSD	0.54

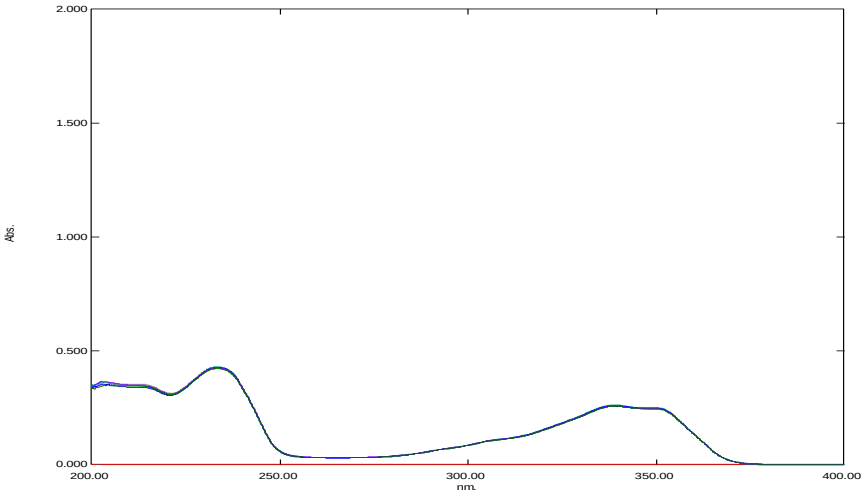


Fig 5: Precision of Upadacitinib

Accuracy:

To test the accuracy of the method, a recovery study was performed by adding the drug standard solution (100 µg/ml) to the pre-analyzed sample solution (100 µg/ml) at three levels: 50%, 100%, and 150%. The drug concentration and recovery (%) were determined by a linear equation. The results obtained are presented in (**Table 5).**

Table 5: Accuracy studies at three concentration level

DRUG	% LEVEL	Volume of sample taken (ml)	Volume of standard added (ml)	Final Concentration in (µg/ml)	Absorbance (n=3)	% Recovery	Mean	SD	%RSD
UPADATINIB	50	0.4	0.2	6	0.39	103	99.5	1.2	1.206
	100	0.4	0.4	8	0.50	97.5			
	150	0.4	0.6	10	0.63	99.9			

Robustness:

The robustness of the method was determined by carrying out the analysis under conditions of varying detection wavelength (±233nm) and checking the effect on absorbance. This method has proven reliable. Robustness results for Upadacitinib are shown in (**Table 6).**

Table 6: Robustness of Upadacitinib

	231nm	233nm	235nm
1	0.27	0.648	0.44
2	0.268	0.65	0.442
3	0.272	0.652	0.44
4	0.269	0.656	0.441
5	0.271	0.643	0.444
6	0.27	0.652	0.445
Mean	0.270	0.650	0.442
SD	0.0015	0.004401	0.0020
%RSD	0.56	0.67	0.47

Ruggedness:

Ruggedness was determined by carrying out analysis by 2 different analysts for 10 µg/ml for 6 times and the respective absorbance was noted and the results were indicated as % RSD. The result of the ruggedness for Upadacitinib is shown in (**Table 7).**

Table 6. Ruggedness of Upadacitinib

S. No	Analyst 1	Analyst 2
Wavelength	233nm	233nm
1	0.658	0.652
2	0.663	0.659
3	0.667	0.664
4	0.665	0.657
5	0.661	0.661
6	0.664	0.656
Mean	0.663	0.658
SD	0.002887	0.003805
%RSD	0.7	0.64

Limit of detection (LOD) and limit of quantification (LOQ):

From linearity data, LOD and LOQ are calculated using the formulas LOD = 3.3*σ/S and
LOQ = 10*σ/S. where σ = standard deviation of the y intercept of linearity equation and
S= slope of the analytical calibration curve. According to UV spectroscopy, the LOD and LOQ were found to be 0.39 µg/ml and 1.17 µg/ml respectively by calculations as in (T**able 7).**

Table 7: LOD & LOQ values of Upadacitinib

Parameter	Values
LOD	0.39
LOQ	1.17

Specificity:

The Proposed method was specific as none of the excipients interfered with the studied drug so the method can be applied for assaying the commercial dosage forms.

CONCLUSION:

The proposed spectrophotometric method developed for determination of Upadacitinib is based on a calibration curve. This method has been tested and is simple, sensitive, precise and accurate. No interference was found with the excipients normally present in tablets. This method has been validated according to ICH guidelines and can be successfully used for routine analysis of Upadacitinib in pharmaceutical formulations.

ACKNOWLEDGEMENT:

We are thankful to the authorities of Sarojini Naidu Vanitha Pharmacy Mahavidyalaya for supporting and encouraging us to carry out the research.

CONFLICTS OF INTEREST: The authors declare that there is no conflict of interests regarding the publication.

ABBREVIATION USED:

API: Active pharmaceutical ingredient, **RSD**:Relative standard deviation, **SD**: Standard deviations.

REFERENCES

1. Raimon S, et al. Upadacitinib for patients with rheumatoid arthritis: a comprehensive review. J Clin Med. 2023;12(5):1734. doi:10.3390/jcm12051734.
2. Martens-Lobenhoffer J, et al. Quantification of the Janus kinase 1 inhibitor upadacitinib in human plasma by LC-MS/MS. J Chromatogr B. 2022;1188:123076. doi: 10.1016/j.jchromb.2021.123076.
3. Kantheti S, Raju RR, Gorumutchu GP. A liquid chromatographic method for the reliable quantification of upadacitinib and its specified impurities. Int J Appl Pharm. 2025;17(4):420–434.
4. Özgür Üstündağ , Erdal Dinç. Validation study of a spectrophotometric binary mixture analysis. J Fac Pharm Ankara. 2021;45(3):545–553. doi:10.33483/jfpau.989819.
5. Inturi R, Raju MD, Rao MVB, Inturi S. Validation of LC-MS/MS method for monitoring of upadacitinib in rat plasma samples. J Pharm Negat Results. 2022;13(2):102–107.
6. Wang MJ, Zhao YH, Fan C, Wang YJ, Wang XQ, Qiu XJ. Development of a UPLC-MS/MS method for the quantitative analysis of upadacitinib in beagle dog plasma and pharmacokinetics study. Drug Des Devel Ther. 2021; 15:4167–4175. doi:10.2147/DDDT.S332282.
7. Baje Syed I, Nannapaneni M. Related substances method development and validation of axitinib, zanubrutinib, and upadacitinib using RP-HPLC and characterization of degradation products by LC-MS/MS. Int J Pharm Investig. 2022;13(1):113–121. doi:10.5530/223097131696.
8. Li J, Chen C, Wang J, Ye Z, Pan L, Liu Z. Simultaneous measurement of upadacitinib and methotrexate by UPLC-MS/MS and its pharmacokinetic application in rats. J Chromatogr B. 2022; 1188:123071. doi: 10.1016/j.jchromb.2021.123071.
9. International Conference on Harmonisation (ICH). Validation of analytical procedures: text and methodology Q2(R1). 2005.
10. Jenis ML, et al. Quantification of the Janus kinase 1 inhibitor upadacitinib in human plasma by LC-MS/MS. J Chromatogr B. 2022;1188:123076.
11. Budak F, et al. Development of a sensitive and simple method for the analysis of upadacitinib in biological samples using two different electrodes. Ionics. 2025; 31:1003–1017. doi:10.1007/s11581-024-05921-7.
12. Sowmya C, et al. Separation and characterization of degradation impurities of upadacitinib by liquid chromatography and high-resolution mass spectrometry. J Chromatogr B. 2024; 1247:124319. doi: 10.1016/j.jchromb.2024.124319.
13. Tachet J, et al. Development and validation of a multiplex HPLC-MS/MS assay for the monitoring of JAK inhibitors in patient plasma. J Chromatogr B. 2023; 1230:123917. doi: 10.1016/j.jchromb.2023.123917.
14. Wu JJ, et al. 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. J Pharmacol Toxicol Methods. 2025; 131:107581. doi: 10.1016/j.vascn.2025.107581.
15. Nimmagadda RL, Gummadi S. A UPLC method development and validation study of upadacitinib and its impurities in extended-release oral tablet dosage forms. Ann Pharm Fr. 2024;82(5):780–791. doi: 10.1016/j.pharma.2024.03.007.

16. Li J, et al. Simultaneous measurement of upadacitinib and methotrexate by UPLC-MS/MS in rats. J Chromatogr B. 2022. doi: 10.1016/j.jchromb.2021.123071.

17. Kashid AM, Kaldate DP. Development and validation of a green stability-indicating RP-HPLC method for upadacitinib: a selective Janus kinase inhibitor. Discover Chemistry. 2025; 2:218. doi:10.1007/s44371-025-00310-z.

18. Rao YV, Chimakurthy J. Quantification of an anti-rheumatic agent: upadacitinib in biological fluid (plasma) by LC-MS/MS. J Pharm Negat Results. 2022;13(Suppl 6):3279–3295. doi:10.47750/pnr.2022.13. S06.442.

19. Mohamed MF, Trueman S, Feng T, Anderson J, Marbury TC, Othman AA. Characterization of the effect of renal impairment on upadacitinib pharmacokinetics. J Clin Pharmacol. 2019;59(6):856–862.

20. Mohamed-Eslam F, Klünder MB, Othman AA. Clinical pharmacokinetics of upadacitinib: review of data relevant to the rheumatoid arthritis indication. Clin Pharmacokinet. 2020;59(5):531–544.

21. Suchitra D, Prashanti C, Sukanya M, Shravan Kumar K. RP-HPLC stability indicating method development and validation for the determination of Lenvatinib in bulk and its application to pharmaceutical dosage form. Int J Food Nutr Sci. 2023;12(1):2578-2594.

22. Suchitra D, Satyanarayana B. A stability indicating reverse phase-HPLC method development and validation for the estimation of rucaparib in bulk and pharmaceutical dosage form. Am. J Anal Chem**.** 2021;12: 96-107. doi:10.4236/ajac.2021.124008.