Original Article

Quantification of an Anti-Rheumatic Agent: Upadacitinib in Biological Fluid (Plasma) By LC- MS/MS

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DOI: 10.47750/pnr.2022.13.S06.442

Abstract

Aim: Upadacitinib is a Janus kinase/signal transducers and activators of transcription (JAKs) of cytoplasmic tyrosine kinase that has been proven to be effective in treating inflammatory conditions and various immunological diseases/disorders, including rheumatoid arthritis. Tofacitinib and ruxolitinib, which are members of this drug's first generation, did not affect JAK1/JAK3 or JAK1/JAK2 receptors specifically due to a lack of subtype selectivity. As far as we are aware, there is currently no method for the accurate quantification of the anti-rheumatic drug upadacitinib in biological fluid.

Material and Materials: To detect upadacitinib in biological fluid, an unique and reliable LC-MS/MS technique must be developed. Using a symmetric C18 column (150 x 4.6 mm, 3.5 m) and isocratic elution with acetonitrile: water (50:50) as the mobile phase, we created a unique bioanalytical approach here. Formic acid was used to bring the mobile phase's pH down to 4.0, and the flow rate was 1 ml/min. The retention time of the medication was discovered to be 3.12 min, and the total run time was set to 7 min.

Results: With a correlation coefficient (r2) of 0.999, the upadacitinib linearity curve was established at concentrations ranging from 12.5 ng/ml to 100 ng/ml. The theoretical plates, resolution, and tailing factor are system suitability parameters found to be within the acceptable criteria. The recovery studies indicated that the developed method can extract the acceptable % amount of drug 100.3%. The matrix effect study indicates there is no effect of matrix on recovery, the result shown as 104.20% and also other validation parameters like precision, LOD, LOQ are within the acceptable criteria.

Conclusion: The created techniques enable a precise, delicate, and consistent analytical process for the estimation of Upadacitinib in biological matrix. The stability studies indicate the drug is stable in different accelerated stability study conditions the results are within limits.

Keywords: Biological Fluid, Development, LC-MS/MS, Upadacitinib, Validation..

1. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic, inflammatory autoimmune condition that can harm joints permanently and result in excruciating pain and incapacity [1,2]. Without the right medical attention, irreversible joint injury and loss can cause serious disability and a lower quality of life. With the development of biological therapeutic techniques including anti-TNF, anti-IL-6, anti-CTLA4 Ig, and anti-CD20 over the past 30 years, the treatment of RA has undergone significant changes [3,4]. Because these medications are more effective, patients have access to a wide range of therapy options [5]. Drugs for the therapy of RA are indeed being developed that target a range of pro-inflammatory pathways, including the family of Janus kinase (JAK) intracellular signaling enzymes [6].

Tyrosine kinases known as JAKs are activated by cytokines when they attach to their cellular receptors in the cytoplasm of those cells [7]. The pathophysiology of RA and other immune-mediated inflammatory diseases, as well as a variety of cytokine signaling pathways involved in regular cellular functions, are heavily reliant on the JAK family, which comprises JAK 1, 2, and 3 and tyrosine kinase 2 (TYK 2) [8].

Oral JAK inhibitors are a member of the targeted synthetic disease-modifying antirheumatic drug class (DMARD) [9,10]. Currently, they are recommended as a treatment choice for RA patients all over the world. These patients continue to show moderate to severe disease activity while receiving conventional synthetic DMARD (CSDMARD) therapy, or they do not respond to biological substance DMARD treatment [11]. It has been shown in the past that inhibiting JAK1 selectively prevents pathogenic cytokine signalling in RA while preserving other signalling pathways essential for physiologically normal functions.

Upadacitinib, a novel selective JAK1 inhibitor, was just approved by the US FDA in 2019 for the treatment of adult patients with moderate to severe RA [12]. Upadacitinib 15 mg significantly improved disease outcomes (remission and LDA) as compared to conventional therapies (methotrexate, adalimumab, and abatacept), while lowering the risk of structural joint damage. Additionally, it demonstrated a favorable benefit-risk profile and met a medical requirement for RA patients [13]. Upadacitinib administration over a prolonged period of time may be beneficial for PSA patients who do not respond to biologic therapy [14,15].

Global prevalence of rheumatoid arthritis (RA) during 1980 to 2019 was increased drastically to report about 460 cases per 100,000 populations [16]. Till date there is no proper treatment to completely eradicate rheumatoid arthritis, but clinical studies indicate that the symptoms are likely to be diminished when treatment begins early with currently used anti-rheumatoid arthritis drugs like NSAIDS, [1,2] corticosteroids, methotrexate, hydroxychloroquine, sulfasalazine, immunomodulatory and cytotoxic agents [17]. The chemical name of Upadacitinib is A bis((3S,4R))-3-ethyl-4 - Tetraazatricyclo-1,5,7,10 [7.3.0.0 2,6] 3, 7, 9, and 11-pentaen-12-yl dodeca-2(6) N-(2,2,2-trifluoroethyl) pyrrolidine 1 carboxamide hydrate, soluble freely in water, has pH range of pKa value as 4.11, molecular formula is C34H40F6N12O3, molecular weight is 380.4 g/mol and tmax of the Upadacitinib is around 2-4 hours [18].

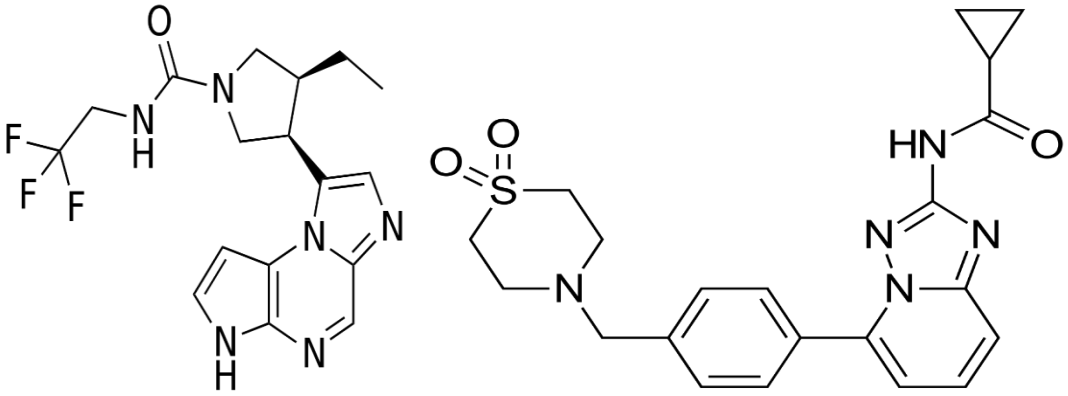


Figure 1 Chemical structures of Upadacitinib & Filgotinib

The Upadacitinib is frequently used in the current treatment of different stages of rheumatoid arthritis since it can specifically be acting on JAK-1 isoform provides drug safety and substitute the other rheumatoid arthritis drugs which need to be used in combination with immunosuppressant and are contraindicated in pregnancy and other conditions [19]. Hence the analytical methods for estimation of Upadacitinib individually (or) in combination are striving for research and industrial purposes.

Upadacitinib is mostly metabolized in vitro by CYP3A and slightly by CYP2D6. Strong CYP3A4 inducers and inhibitors have a considerable impact on the AUC and Cmax of Upadacitinib. Therefore, it is advised to use upadacitinib CYP3A4 inducers should not be combined with strong CYP3A4 inhibitors in patients on long-term therapy [20]. To examine the drug-drug interactions of upadacitinib, it is important to establish a quantitative analytical approach. The literature review revealed that there are no existing analytical approaches for Upadacitinib quantification in biological fluids, bulk pharmaceutical dosage forms, and pharmaceutical dosage forms [21]. The current study's objective is to develop and validate a reliable LC-MS/MS technique for the routine analysis of upadacitinib in bodily fluids in accordance with USFDA criteria [22].

A quick, sensitive, and precise approach for figuring out the drug concentration in biological samples was the LC-MS/MS. Upadacitinib human pharmacokinetics have been reported, however the explanation of how to measure upadacitinib levels in the blood is lacking [23]. The entire methodological validation of the plasma upadacitinib concentration detection is not currently documented. Filgotinib was chosen as the internal standard in this study, and we developed and validated a quick and

accurate LC-MS/MS assay to quantify upadacitinib in rat plasma [24]. (IS, Figure 1B). Additionally, the pharmacokinetics of upadacitinib in diverse plasma samples may be effectively studied using the developed and validated LC-MS/MS method.

2. Material and Methods

2.1 Reagents and chemicals

Upadacitinib samples were provided from Biocon, Bangalore as a gift sample. Solvents, acetonitrile and methanol of analytical grade were purchased from the Mumbai-based Merck Chemical Division. The Quantification investigation employed LC grade water that was gathered from the Milli-Q water system.

2.2. Instrumentation:

Chromatographs are performed by the using of Alliance Waters e 2695 high performance liquid chromatography (HPLC) having ultra-speed automatic sampling technique with column oven, degasser with attachment of QTRAP 5500 triple quadrupole mass spectrometer and software as SCIEX.

2.2.1. Preparation of Upadacitinib Stock Solutions

Upadacitinib, accurately weighed at 5 mg, was transferred to a graduated flask with a volume of 10 mL, and the diluent was added. (Acetonitrile: Water (50:50)) to get the primary stock solution. The prepared stock solution was further diluted by serial dilution method using the diluent to get the different concentrations of upadacitinib.

Preparation of Standard working solution of Upadacitinib: Transfer 500µL of standard stock solution to 2 mL of centrifuge tube and add 200µL of plasma, 500µL of internal standard, 300µL of acetonitrile and 500µL of diluent. The prepared sample was then centrifuged at 4000 rpm using a cooling centrifuge for 20 min. A careful collection of the supernatant solution into an HPLC vial was made. The different aliquots of 12.5 ng to 100 ng/mL of upadacitinib were prepared and centrifuged for 15 to 20 minutes at 4000 rpm. taken supernatant solutions are injected into the HPLC system.

Internal Standard Stock Solution (200ng/ml) Preparation: A precisely weighed 5 mg dose of Filgotinib was transferred to a graduated flask with a volume of 10 mL, and the diluent was used to dilute it. There were more dilutions done to get different concentrations of 200 ng/mL by serial dilution method with the diluent.

2.2.2. Detection of Wavelength: Determination of λ max

suitable volume Upadacitinib standard stock solution was diluted to a target concentration of 5 g/ml using distilled water after 0.5 ml was put into a 10 ml volumetric flask. The final product was scanned in the UV range (200–400 nm). Within the range of upadacitinib it shows absorbance maximum at 283 nm.

2.2.3. Preparation of Plasma matrix: The plasma samples used for the study were directly purchased from the local blood banks. 3 mL of plasma sample was aliquot from the stock which is stored at -20oC after thawing. 200 µL each was collected in separate eppendorf tubes and the different concentration of pure drug solution was spiked into the plasma and mixed well.

2.2.4. Extraction procedure from Plasma: Protein precipitation extraction method using chilled acetonitrile was followed to extract the drug upadacitinib from the plasma matrix. To about 200 µl of plasma, 500 µL of diluent was added and vortexed for 2 min. To this 300 µL of chilled acetonitrile was added in order to precipitate every protein. The supernatant solution was collected into the HPLC vial and injected into the HPLC after the sample had been centrifuged at 4000 rpm for 15 to 20 minutes. for an estimation drug extracted from plasma.

2.2.5. Chromatographic Specifications: Separation was performed using a Symmetry C18 column (150x4.6mm, 3.5m) and an isocratic elution with a buffer containing 1mL of formic acid in 1 litre of water, as well as a mixture of buffer and acetonitrile in a 50:50 ratio as the mobile phase with a flow rate of 1mL/min at room temperature.

2.2.6. Bio analytical method development : An analytical procedure should be validated in order to demonstrate the effectiveness and dependability of the method and, consequently, the level of confidence that can be placed in the results. Additionally, in order to support the registration of a novel medicine or the reformulation of an existing one, all bioanalytical procedures must be verified. It should be emphasized that the first validation is simply the start, since a method should be continuously watched while being used to make sure it functions as intended. By using specialized laboratory studies, validation involves proving that the performance characteristics of the methods are appropriate and trustworthy for the intended analytical applications.

2.2.7. Validation of the developed bioanalytical method:

Using Filgotinib as the internal standard (IS) and the isocratic elution procedure, the desired concentrations of the drug samples were produced from the primary stock solution. The improved bioanalytical approach was verified for a number of validation criteria in accordance with USFDA requirements.

System suitability and selectivity

The standard solution containing Upadacitinib (50 ng/mL) and Filgotinib (50 ng/mL) was injected into the HPLC six times to determine the system suitability parameters in order to assess whether the proposed bioanalytical technique is appropriate for its intended use. At LLOQ values, Upadacitinib selectivity was determined. The calibration curve and three duplicates of LLOQ were processed together.

Specificity

To verify whether the developed bioanalytical method can specifically resolve al the analytes, in three replicates of drug solution, IS, blank plasma, spiked plasma and diluents that were injected into HPLC.

Linearity

Upadacitinib calibration curves were constructed by regressing the ratio of analyte peak regions to analyte concentrations. After the correct dilutions, Upadacitinib was created at the necessary concentrations (12.5, 25, 37.5, 50, 62.5, 75.0, and 100 ng/mL) from the primary stock solution (5000 ng/mL) solution. It was discovered that Upadacitinib LLOQ was 10 ng/mL.

Precision

Precision was used to gauge how well the established bioanalytical approach would produce reproducible results. By injecting known drug concentrations into blank plasma matrices, precision was achieved for upadacitinib at low quality control (LQC), middle quality control (MQC), and high quality control (HQC) levels, i.e. at 12.50, 25, 37.50, 50, 62.5, 75, and 100 ng/mL. To evaluate the intra-day and inter-day precision, six replicates of each sample were injected at three different quality control levels.

Accuracy

By injecting six copies of each sample into HPLC at each of the three QC levels—LQC, MQC, and HQC—the developed method's accuracy was evaluated. Using the linear equations, the peak area ratio of the drug/IS was estimated, and the actual concentrations were then back-calculated. By determining the difference between the actual and theoretical concentrations, accuracy was assessed.

Recovery

The degree of drug recovery from biological matrices was assessed by comparing the % recovery from extracted samples to that from unextracted samples. Six replicates of spiked samples at the LQC, MQC, and HQC levels were run through the HPLC for this. Injections of unextracted material with same quality control levels showed 100% recovery as well.

2.2.8. Methodology for Analysis

Extraction of blank plasma samples along with the extraction of drug spiked plasma sample solutions were inserted into the HPLC chromatographic system in order to calculate the drug peak's potential interference with the plasma peak. Calculating the peak area ratio of the internal standard to upadacitinib-spiked plasma sample led to the establishment of the linearity curve. IS to upadacitinib to obtained the linearity Curve. An ESI (Electron spray ionization) has been utilized to study upadacitinib. This approach was successfully applied, to explore upadacitinib with its IS extracted from Human biological (plasma) using protein precipitation method.

3. Results and Discussions

3.1. Reagents and Chemicals:

The study was conducted with only analytical-grade chemicals and reagents.

3.2. Bioanalytical method development: The bioanalytical method was developed by spiking drug to the plasma and extraction of drug from the plasma by protein precipitation method.

The drug extracted from samples were centrifuged and supernatant was collected were injected into the HPLC for the estimation of drug.

3.3. Validation of the developed bioanalytical method:

System suitability

Determine the by examining a set of Thesaurus before the analytical run, the instrument performance is assessed. The %CV for Upadacitinib and IS results are found to be as 0.48. Hence the system suitability parameters were correlated with the USFDA Guidelines.

Table 1 System suitability of Upadacitinib

Upadacitinib MQC (50ng/ml)	Area of sample (cps)	Retention Time (min)	Ratio
*Mean ± SD	3.23500 ± 0.012	3.114 ± 0.003	0.9346 ± 0.004
%CV	0.38	0.08	0.48

*Mean n= 6 replicates

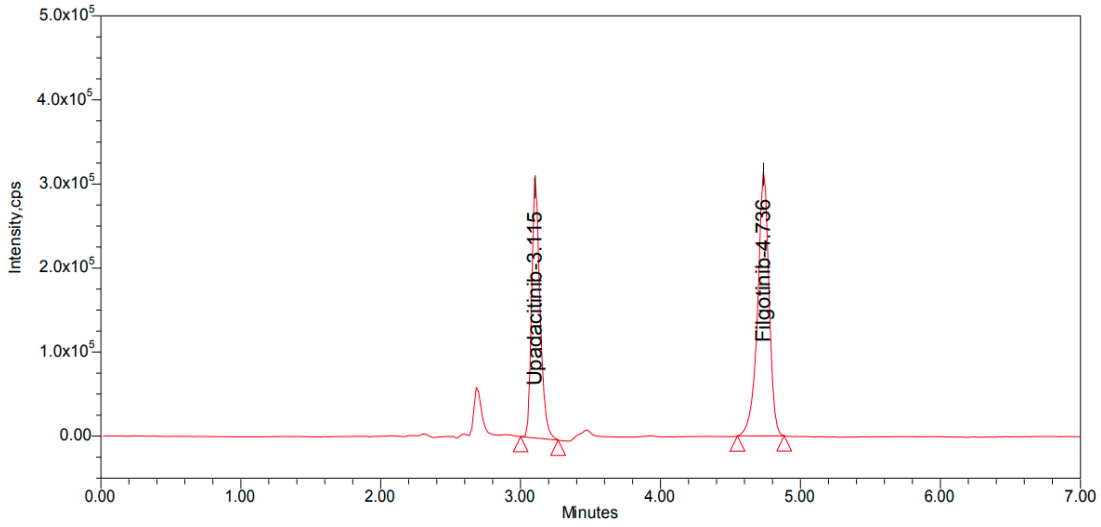


Figure 2 Chromatogram of System suitability

3.2 Auto sampler carryover

Auto sampler carryover responses in alternate injections were found to be <20%. Hence the carryover parameter results were correlated with the USFDA Guidelines.

Table 2 Autosampler carryover of Upadacitinib

Sample ID	Peak area	Recovery
	Drug	Drug
Un extracted samples		
RS	0	N/A
HQC		0.00
RS	0	
LLOQ		N/A
Extracted samples		
Blk	0	N/A
HQC		0
Bulk	0	
LLOQ		N/A

3.3 Specificity and screening of biological matrix

There are no interfering peaks were found out of six different random blank Human Plasma samples at the elution times of either upadacitinib or ISTD. Hence the specificity results were correlated with the USFDA guidelines.

Table 3 Specificity results of upadacitinib

S. No.	Upadacitinib	Peak area	% Interference	
		Drug	Drug	Pass/ Fail
1	LLOQ 1 (5ng/ml)	36900	0	Pass
2	LLOQ 2 (5ng/ml)	36100	0	Pass
3	LLOQ 3 (5ng/ml)	36500	0	Pass
4	LLOQ 4 (5ng/ml)	36700	0	Pass
5	LLOQ 5 (5ng/ml)	36300	0	Pass
6	LLOQ 6 (5ng/ml)	36400	0	Pass

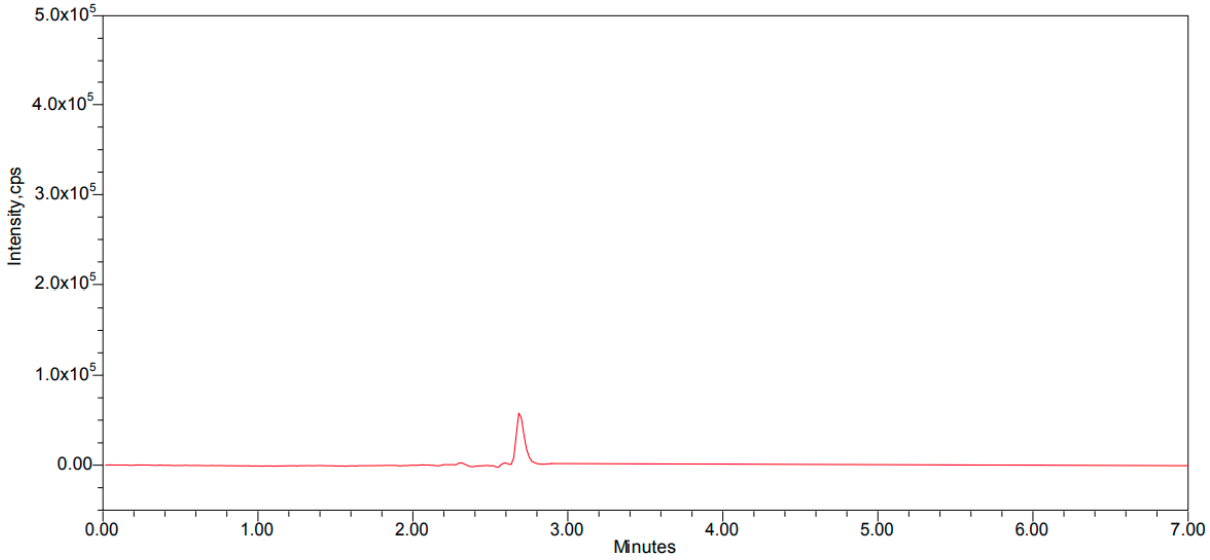


Figure 3 Specificity Chromatogram of Placebo

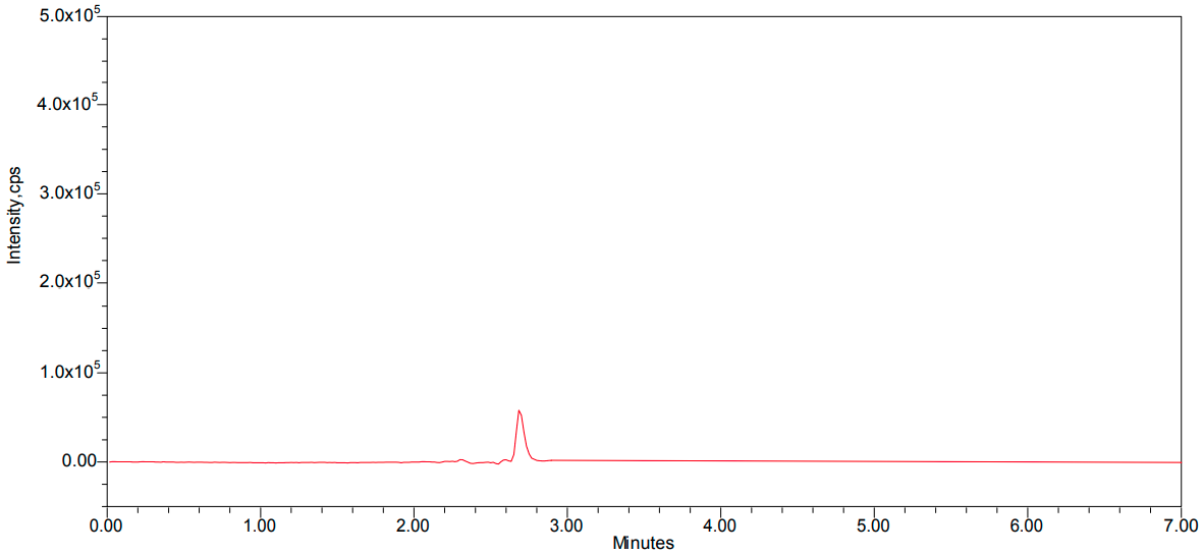


Figure 4 Specificity Chromatograph of Blank

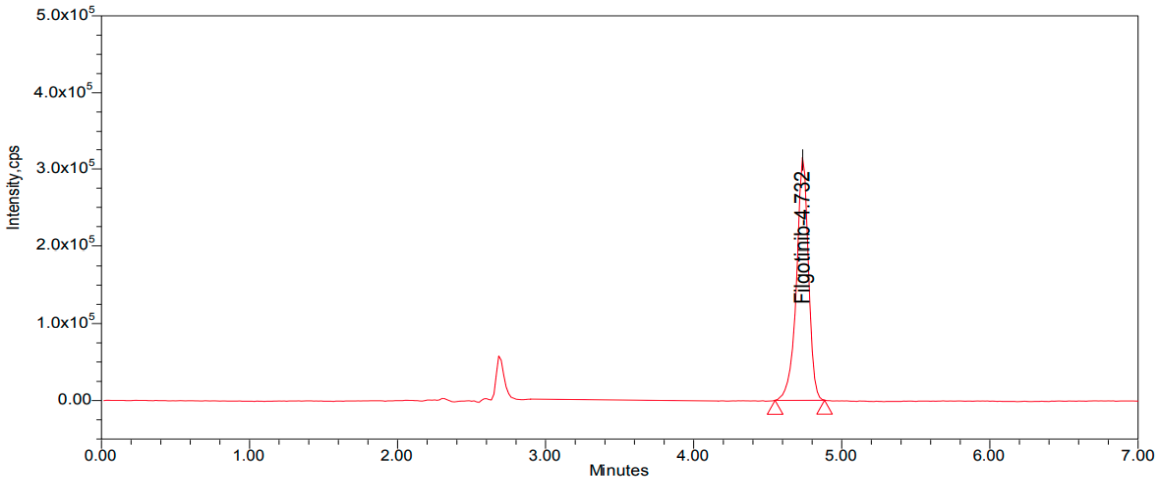


Figure 5 Specificity Chromatograph of ISD

3.4 Sensitivity

The %CV & mean accuracy was found to be as 0.83%, 112.83%within acceptance limits of USFDA guidelines.

Table 4 Sensitivity Results of Upadacitinib

Parameter	LLOQ
	Nominal Concentration(ng/ml)
	5.0
	Analyte peak area
n	6
Mean	$0.365x10^5$
SD	0.0030 ± 0.83
% Mean	112.83%

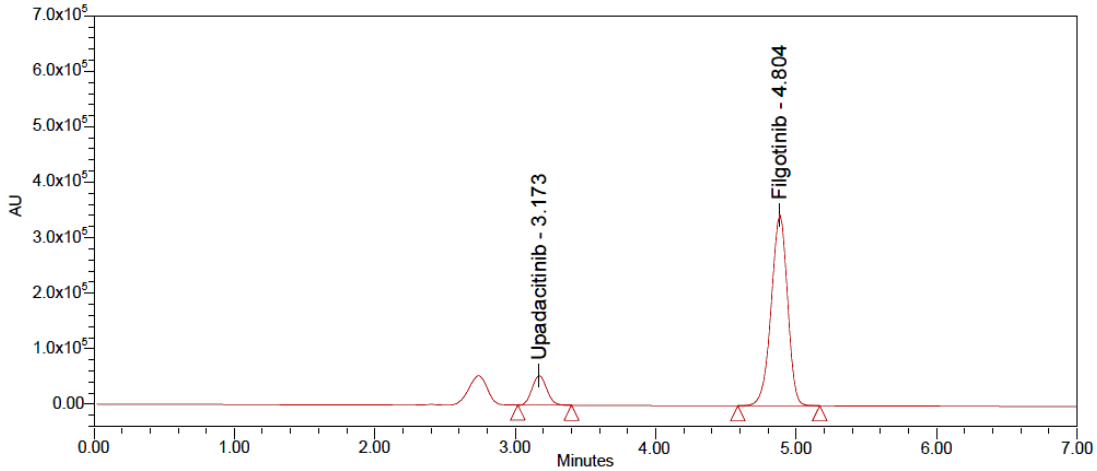


Figure 6 Sensitivity Chromatogram of LLOQ

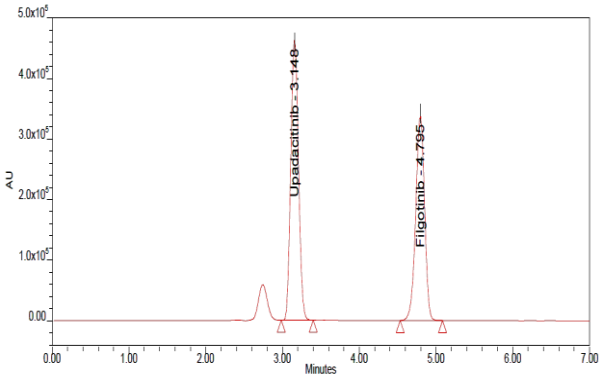
3.5 Matrix effect

Matrix effect of biological fluid concentrations over the ionization of analytes were analysed by compared response of post-extracted sample and Std MQC samples (50 ng/ml of Upadacitinib) response of analytical samples at equal concentrations. This method was analysed by chromatographic screening of Human Plasma.

Table 5 Matrix effect Results of Upadacitinib (HQC-75 ng/ml, LQC-25ng/ml)

S.No.	HQC	LQC
	Concentration Ranges (ng/ml)	
	75.0	25.0
	Analyte peak area	
n	18	18
Mean	484900	173200
SD	0.013 ± 0.26	0.013 ± 0.74
% Mean Accuracy	99.93%	107.08%

The matrix effect for the different concentrations of biological samples were found be as for HQC is 99.93% and LQC is 107.08%. Hence the results were correlated with the acceptance criteria of USFDA guidelines.



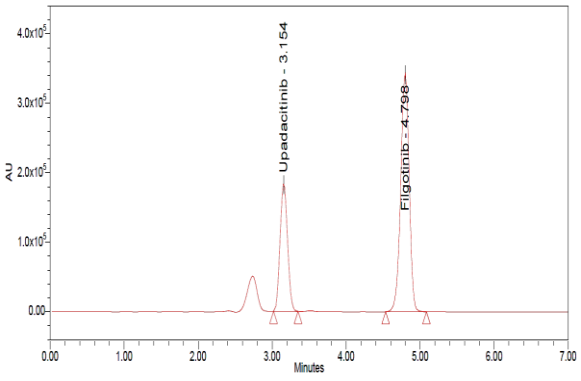


Figure 7 Matrix Effect Chromatogram of LQC Figure 8 Matrix Effect Chromatogram of LQC

3.6 Linearity

The standard curves of linearity ranges from 12.5-100 ng/mL of Upadacitinib was found to be as 0.999. These analytical samples were analyzed by using the proportion between analyte peak area and IS peak area. Graph was plotted against to plasma concentrations R^2 was found to be as 0.999.

Table 6 Linearity results of Upadacitinib

Final conc. in ng/ml	Area response ratio
12.50	0.255
25.00	0.503
37.50	0.724

50.00	0.937
62.50	1.169
75.00	1.400
100.00	1.881
Slope	0.0184
Intercept	0.02763
R^2 Value	0.99961

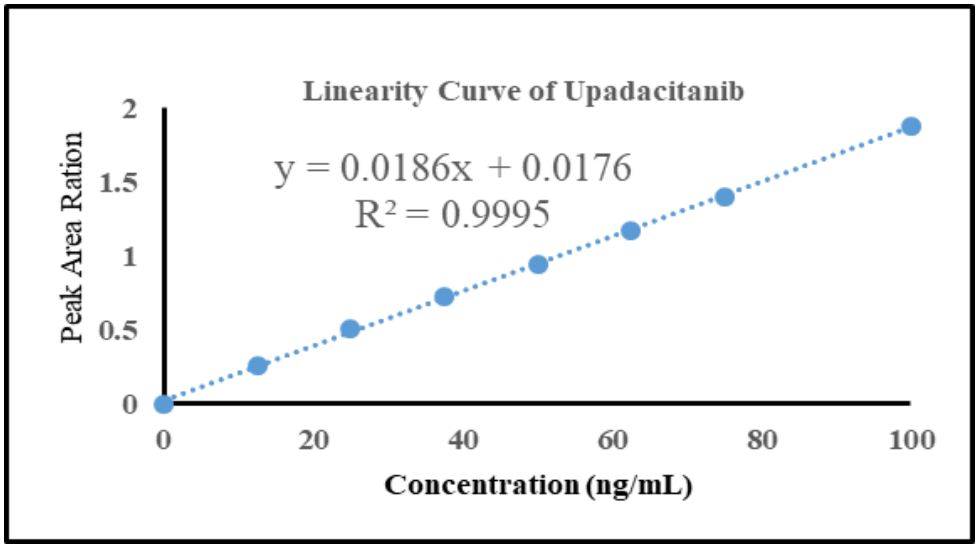


Figure 9 Calibration graph of Upadacitinib

3.7 Precision and accuracy

Six replicates at four distinct QC levels were utilised to analyse repeatability and reliability. Reproducibility was determined by analyzing the four different samples on individual trails. Hence the obtained results were corresponding with USFDA Validation parameters.

Table 7 Precision Results of Upadacitinib

Sample Upadacitinib	HQC	MQC	LQC	LLQC
	Nominal Concentration (ng/ml)			
	75.0	50.0	25.0	5.0
	Analyte peak area			
n	6	6	6	6
Mean	$4.857x10^5$	$3.236x10^5$	$1.729x10^5$	$0.369x10^5$
SD	0.014 ± 0.29	0.015 ± 0.46	0.014 ± 0.82	0.016 ± 4.43
% Mean Accuracy	100.09%	100.03%	106.89%	114.06%

Both inter and intra precision values of HQC, MQC, LQC, LLQC were found to be satisfactory and correlates with the guidelines of USFDA.

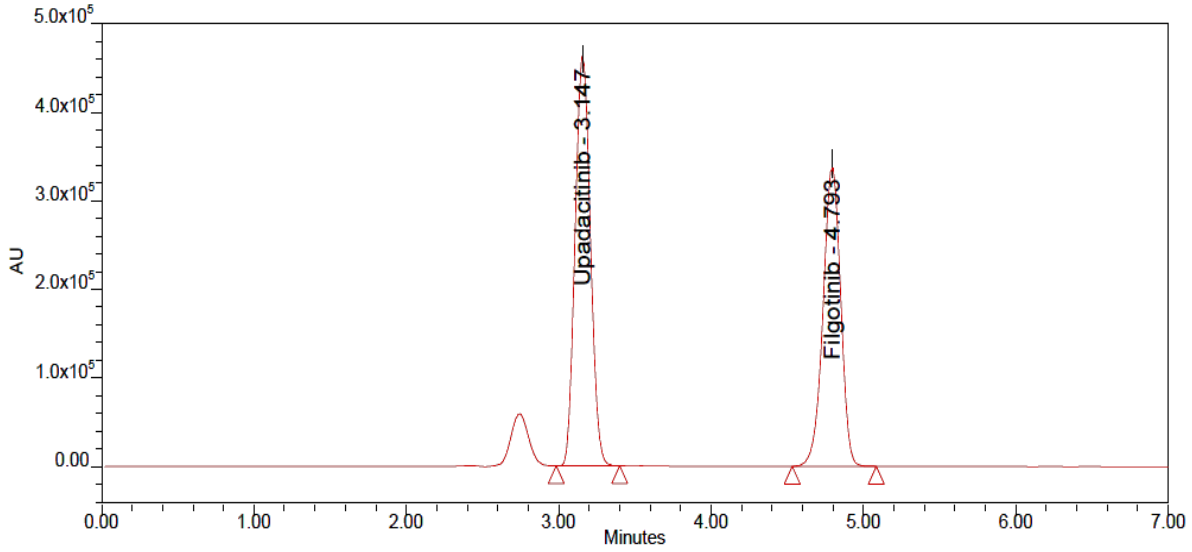


Figure 10 Chromatogram of Precision and accuracy of HQC

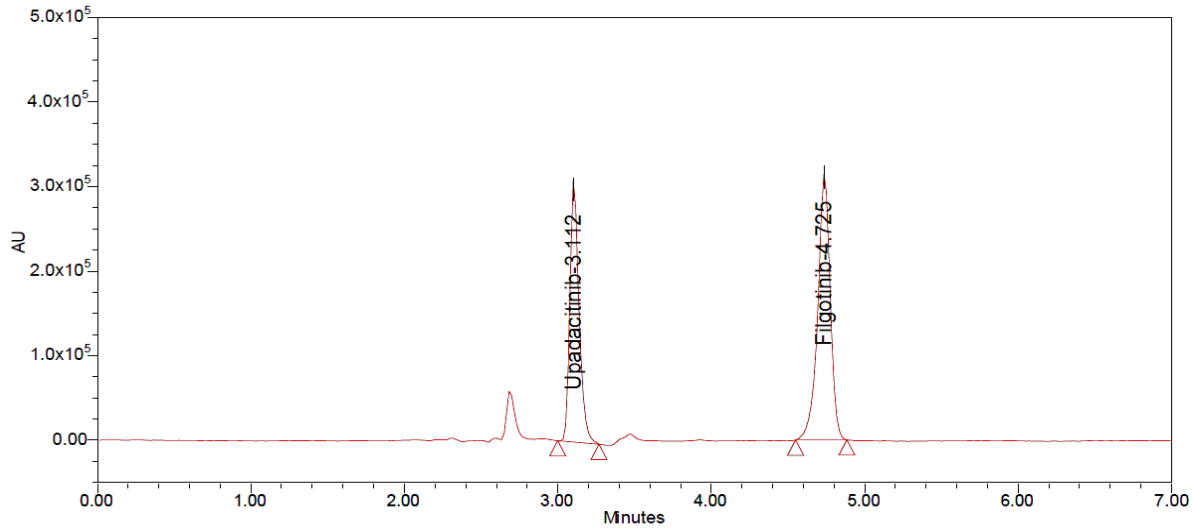


Figure 11 Chromatogram of MQC

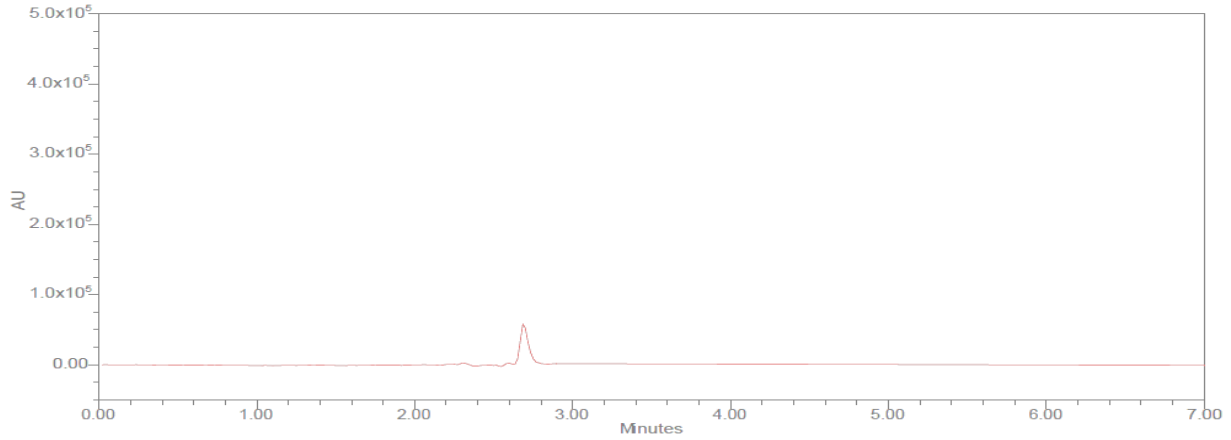


Figure 12 Chromatogram of Blank

Table 8 Ruggedness on precision accuracy of Results of Upadacitinib

S.No	HQC(75 ng/ml)	MQC (50ng/ml)	LQC (25ng/ml)
	Nominal Concentration (ng/ml)		
	75	50	25
	Analyte peak area		
n	6	6	6
Mean	485600	324300	174800
SD	0.016 ± 0.33	0.013 ± 0.41	0.011± 0.65
% Mean Accuracy	100.07%	100.25%	108.07%

Within the batch and in between the batch precision for three different concentration samples were found to be meets with USFDA Guidelines Criteria.

Table 9. LOD and LOQ Results

Drug Name	LOD (S/B) Value	LOQ (S/B) Value
Upadacitinib	4	25

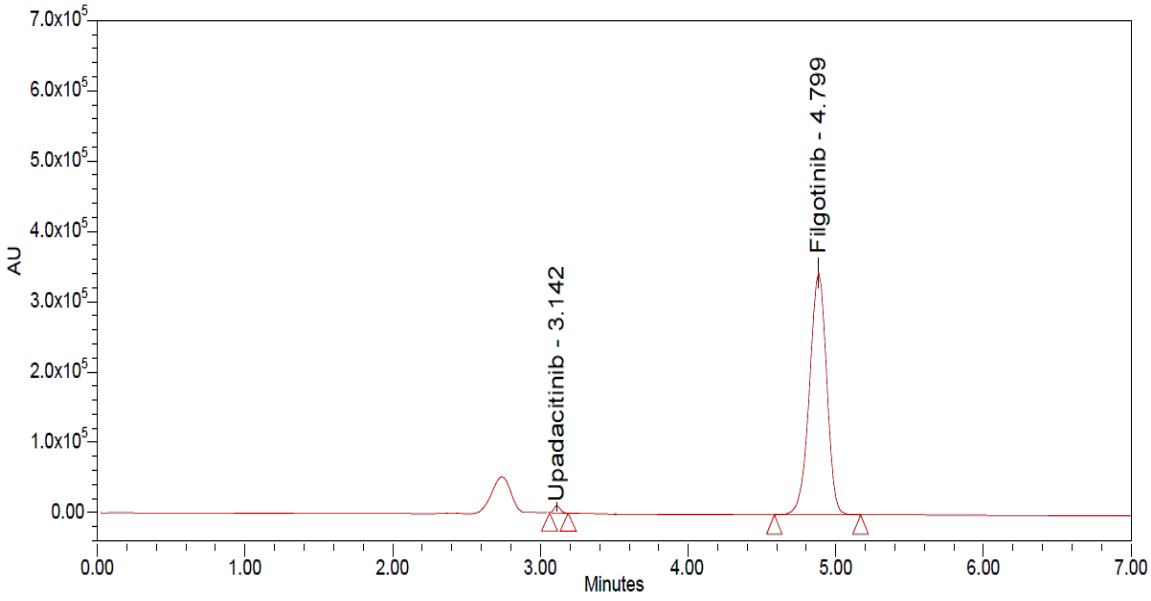


Figure 13 LOD Chromatogram

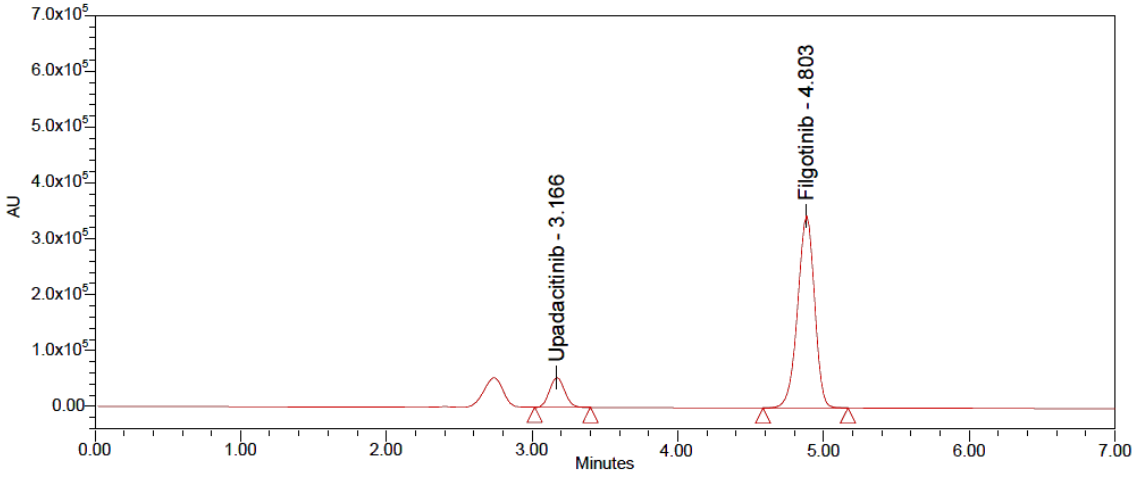


Figure 14 LOQ Chromatogram

3.8 Recovery of Analyte

The recovery of drug and IS were Calculated at three concentration levels by comparing its response in six replicate samples with standard solution responses. Analyte was recovered from a sample matrix, and the analytical response between the amount of analyte added and that estimated from the sample matrix was compared. Extraction was carried out by using solvent as mobile phase.

Table 10 Recovery of analyte of Upadacitinib

Replicate Number	HQC (75 ng/ml)			MQC (50 ng/ml)			LQC (25 ng/ml)		
	Formulation	standard	Matrix factor	Formulation	standard	Matrix factor	Formulation	standard	Matrix factor
n	6	6	6	6	6	6	6	6	6
Mean	$4.853x10^5$	$4.965x10^5$	0.977	$3.245x10^5$	$3.371x10^5$	0.9627	$1.735x10^5$	$1.841x10^5$	0.9421
SD	0.013 ± 0.27	0.011 ± 0.23	0.001 ± 0.11	0.014 ± 0.44	0.011 ± 0.33	0.005 ± 0.51	0.01 ± 0.55	0.013 ± 0.72	0.004 ± 0.47
%Mean recovery	100.01%	102.32 %	-	100.31%	104.20%	-	107.26%	113.82%	-

% CV of each recovery analyte was should be within the acceptance criteria.

3.9 Ruggedness on reinjection reproducibility

The %CV for Upadacitinib were found to be 0.36%-1.13%. Hence it passed the Ruggedness on reinjection reproducibility.

Table 11 Ruggedness on reinjection reproducibility Results of Upadacitinib

S.No	HQC (75ng/ml)	MQC (50ng/ml)	LQC (25ng/ml)
	Nominal Concentration (ng/ml)		
	75	50	25
	Analyte peak area		
n	6	6	6
Mean	485500	324800	173200
SD	0.022 ± 0.44	0.009 ± 0.28	0.014 ± 0.8
% Mean Accuracy	100.05%	100.40%	107.08%

Ruggedness on reinjection reproducibility results are found to be as satisfactory.

Bench Top Stability %CV of HQC and LQC, mean accuracy for Upadacitinib was passed the Bench top stability.

Table 12 On Bench Stability of Upadacitinib

Replicate Samples	HQC	LQC	MQC
	Nominal Concentration(ng/ml)		
	75.0	25.0	50.0
	Analyte peak area		

n	6	6	6
Mean	485500	175300	324800
SD	0.016 ± 0.34	0.009 ± 0.54	0.013 ± 0.41
% Mean Accuracy	100.05%	108.38%	100.40%

We found that bench top stability studies for three concentrations of HQC, MQC & LQC were satisfies the USFDA Norms.

3.10 Autosampler Stability.

Auto Sampler Stability The %CV of HQC, MQC, LQC and mean accuracy for Upadacitinib passed the

Table 13 Auto Sampler Stability of Upadacitinib

Replicate No.	HQC	MQC	LQC
	Nominal Concentration (ng/ml)		
	75.0	50.0	25.0
	Analyte peak area		
n	24	24	24
Mean	486400	324300	175000
SD	0.012 ± 0.26	0.015 ± 0.47	0.016 ± 0.89
% Mean Accuracy	100.24%	100.25%	97.61%

We find out that auto sampler stability studies were accepted within the recommended criteria of USFDA guidelines.

Freeze Thaw The %CV and mean accuracy for Upadacitinib passed the freeze thaw stability.

Table 14 Freeze Thaw Stability of Upadacitinib

Replicate No.	HQC	LQC	MQC
	Nominal Concentration(ng/ml)		
	75.0	25.0	50.0
	Analyte peak area		
n	6	6	6
Mean	484800	174500	325600
SD	0.014 ± 0.29	0.016 ± 0.94	0.009 ± 0.27
% Mean Accuracy	99.91%	107.88%	100.65%

Freeze thaw stability studies of UPADACITINIB Was found be as for three different concentrations, serially, 99.91 % (HQC), 107.88 % (LQC), 100.65% (MQC). Correlates with the USFDA Guidelines.

4. Summary and Conclusion

Aim and objective of this work was well developed, simple, precise, inexpensive & robust method to analyse the UPADACITINIB by the utilization of LC-MS/MS internal standard as Filgotinib shows less run time never before reported with run time of 7.0 min and our drug was eluted at 3.115 mins. With linearity range of 7 replicates from 12.5 – 100 ng/ml with regression coefficient of r2 is 0.999. And all the analytical validation parameters were correlates with the USFDA guidelines.

Abbreviations:

ULOQ stands for Upper Limit of Quantity, LQC stands for low quality control, MQC for middle quality control, HQC for high quality control, LLOQ for lower limit of quantity, and IS for internal standard.

Acknowledgements

The authors acknowledge the support of the experimental and analytical work provided by Vignan's Foundation for Science, Technology & Research (deemed to be a university) and Shree Icon Laboratories in Vijayawada, India.

Conflict of interest

Authors assure that there is no conflict of interest.

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