RESEARCH PAPER

Bioanalytical Method Development and Validation of Ritlecitinib in Rat Plasma by UPLC and its Application to Pharmacokinetic Study

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ABSTRACT

Background: Ritlecitinib is used for the treatment of severe alopecia areata. Ritlecitinib is a kinase inhibitor which inhibits Janus kinase 3 and tyrosine kinase. Thus, there is a greater interest in developing a unique and reliable UPLC approach for the determination of Ritlecitinib.
Objective: To establish a novel quick and sensitive UPLC technique for the concurrent quantification of Ritlecitinib in rat plasma with Nilotinib as the internal standard.
Method: Separation was carried on column Waters Symmetry C18 column, 150mm x 4.6mm, 3.5µm using an isocratic elution with a buffer containing 0.1% TFA was dissolved in 1 liter of HPLC grade water and adjust its pH-2.5 with Formic acid in the ratio of 60:40 ACN and Buffer as mobile phase with 0.2 mL/min flow rate at ambient temperature.
Results: Analysis was carried out within 3 minutes over a good linear concentration range from 250ng/mL to 7500 ng/mL (r2 = 0.99994) for Ritlecitinib .The extraction recoveries and matrix effect of Ritlecitinib at different QC concentration levels, precision and recovery study results are within the acceptable limit. This procedure has been effectively implemented, examining Ritlecitinib and, along with its internal standard Nilotinib which were extracted from rat plasma utilizing liquid-liquid extraction.
Conclusion: The medications demonstrated stability throughout the stability tests in accordance with USFDA criteria, as the validated technique effectively facilitated the pharmacokinetic investigations of the two drugs.
Keyword: *UPLC, Ritlecitinib, Nilotinib, Bioanalytical Method, development, validation, Rat plasma.*
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1. INTRODUCTION

One medicine that helps with severe alopecia areata (hair loss) [1, 2] is Ritlecitinib, which is marketed as Litfulo. The kinase inhibitor Ritlecitinib inhibits the activity of Janus kinase 3 [3, 4] and tyrosine kinase [5]. Headaches, diarrhoea, acne, rashes, and eczema [6, 7] are among the most often reported adverse effects. Other common side effects include fever, mouth ulcers, disorientation, shingles outbreak [8, 9], and aberrant findings in certain laboratory test results. One member of the kinase family that is extremely selectively inhibited by hepatocellular carcinoma (TEC) [10, 11] and Janus kinase 3 (JAK3) is Ritlecitinib (PF-06651600). Oral Ritlecitinib is the pioneering drug in its category. A serine residue is found at Cys-909 of JAK3, a location that Ritlecitinib binds to covalently. This renders Ritlecitinib an irreversible JAK3 inhibitor with a high degree of selectivity. Some kinases, including those in the TEC kinase family, include cysteines at positions similar to JAK3's Cys-909. It has been proposed that Ritlecitinib blocks cytokine signalling and the cytolytic activity of T cells, two factors involved in the pathological progression [12, 13] of alopecia areata,

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due to its dual action towards JAK3 and the TEC kinase family.

In recent years, only a limited number of studies have been published about the determination of Ritlecitinib (Fig.1) and Nilotinib (Fig.2) utilizing UPLC. We noted several challenges, including extended runtimes and the high costs associated with sample and mobile phase preparations in prior methodologies. Our created technique is validated according to USFDA criteria, has a reduced run time, enhanced precision, lower cost, favorable linear calibration curves, and an outstanding recovery rate.

The bioanalytical test was effectively utilized in the pharmacokinetic research of Ritlecitinib. However, to date, no unique techniques have been developed for the determination of Ritlecitinib.

This study aimed to (a) develop and validate a specific and sensitive UPLC method for the quantification of Ritlecitinib in rat plasma, and (b) evaluate the pharmacokinetics of Ritlecitinib following intravenous administration of test extract in rats.

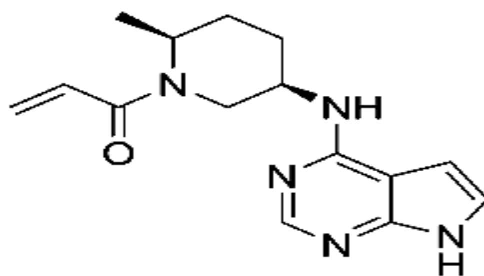

Fig. 1. Structure of Ritlecitinib

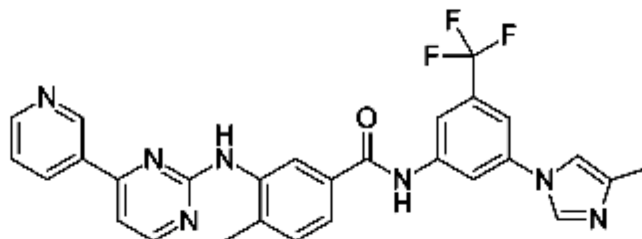

Fig. 2. Structure of Nilotinib

2. MATERIALS AND METHOD

2.1 Chemicals and Materials

Glenmark Life Sciences Ltd. of Mumbai supplied the Ritlecitinib samples used as the reference sample. The Merck chemical division in Mumbai supplied all of the chemicals, including HPLC grade acetonitrile and HPLC grade methanol. All of the water used in the experiment was of the HPLC quality, and it was sourced from the Milli-Q water purification system.

2.2 Instrumentation

Chromatography was performed with waters Acquity UPLC with class Empower-2 software.

3. EXPERIMENTAL

3.1 Preparation of Ritlecitinib stock solution

Take 5mg of the Ritlecitinib working standard into a 10ml volumetric flask and 7ml of diluents and Sonicate for 10 minutes to dissolve the contents completely and make up to the mark with diluent. Further diluted 4 ml to 10ml with diluent. Further dilution by taking 1 ml into 10ml volumetric flask and made up to the mark with diluents.

3.2 Preparation of Internal Standard (Nilotinib) Stock Solution

For sample preparation, aliquots of 200 µl of rat plasma specimens were supplemented with 500 µl of the internal standard (IS) working solution. Subsequently, 300 µL of buffer and methanol were vortexed for 15 minutes. The samples were then centrifuged at 5000 rpm for 15 minutes. The supernatant was split, collected, and filtered through a 0.45 µm nylon syringe filter into a vial before being injected into the LC system.

3.3 Preparation of plasma sample solution

For sample preparation, aliquots of 200 µL of rat plasma specimens were spiked with a working solution containing 500 µL of internal standard (IS), 500 µL of standard stock. After that, 300 µL of acetonitrile and 500µl of diluent were mixed in a vortex for 20 mins, and then the specimens were centrifuged at 4000 rpm for the same amount of time. A supernatant managed solution was then divided, collected, and filtered using a 0.45 nylon syringe filter before being injected into the UPLC system.

3.4 Validation of Bio analytical Method

3.4.1 Matrix Effect

The Effect matrix for Levodopa and Benserazide was assessed by comparing the peak zone proportion in the post-extraction plasma sample with that from six distinct medication-free plasma samples and recovery samples.

3.4.2 Recovery

Experiments were conducted in triplicate using six distinct plasma lots at MQC levels, achieving acceptable accuracy (percent CV) of ≤15%. The extraction efficiencies of

Levodopa and Benserazide were determined by analyzing six repetitions at each concentration of quality control, with recovery levels evaluated by comparing the prominent features of each guidelines to the non-extracted peak regions of standards.

3.4.3 Precision and Accuracy

The evaluation was conducted at lower quantification limit (LLOQ), low quality control (LQC), medium quality control (MQC), and high quality control (HQC) levels using replication analysis of quality control specimens (n=6). The coefficient of variation (CV) should be below 15%, except for the lower limit of quantification (LLOQ), when it should be below 20%.

3.4.4 Stability

Stability of stock solution was achieved by comparing the area response of the analyte in the stability samples with the region response of the specimen prepared from the fresh stock solution. Plasma stability experiments were conducted at concentration levels of LQC and HQC using six replicates at each dose. Analyte was considered to be steady if the shift is less than 15 percent according to the guidelines of the USFDA. For 24h, stability of spiked rat plasma samples stored at room temperature (bench top stability) was assessed. Stability of spiked rat plasma deposited in auto sampler 2-8 °C (auto sampler stability) was assessed for 24h. The stability of the auto sampler was led by comparing the extract plasma samples which were injected immediately with the samples which were re-injected at 2-8 °C for 24h after storage in the auto sampler. The durability of the freeze-thaw was achieved by contrasting the durability samples that were frozen at -30°C and three times thawed, with freshly spiked quality control samples. The freeze-thaw stability assessment used six aliquots each of the LQC and HQC concentration ranges. The concentration obtained after 24h was compared with initial concentration for long-term stability assessment.

4. RESULTS

4.1 Bioanalytical Method development

For the development of plasma calibration standards and the control of the specimens, rat buffered blank plasma was obtained. Biocon, Bangalore, provided plasma samples for the specificity check experiment.The goal was to find a way to measure Ritlecitinib LQC level in rat plasma that would be accurate, precise, and specific enough. Optimization of the following parameters was connected with the study.In the following sections, explain a functional strategy designed for improving analytical efficiency that organizes process selection, implementation, and optimization process.

Ritlecitinib chemical properties were supported by the mobile process. Peak elution would be influenced by the high ratio of organic modifiers and buffers used. In this case, the solubility of the buffer in the organic process will also act as an important role in the analytical system.The standard UPLC technique for analyzing pharmaceutical raw materials is different from the bioanalytical UPLC method. In most situations, the matrix chemicals co-elute with the analyte, which poses a problem to UPLC scientists when using the matrix for bio-analysis. To differentiate the interference peak from the analyte, several stationary stages were used, including cyano, amino, and nitrile. All chromatographic parameters, with the exception of the column, were maintained constant throughout the procedure. Following the determination of the internal norm, the retention duration of Ritlecitinib with its IS was used to inform the final tuning of the mobile phase composition and buffer concentration. To ensure proper peak separation during elution of Ritlecitinib and its IS, the last mobile step was used.Different flow rates are tested after the mobile phase composition is optimized to ensure proper RT, peak asymmetry and resolution of drug and it IS. Based on the RT, proper peak asymmetry, and resolution, the finalized flow rate is determined. The bio-analytical procedure was verified in by ICH guidelines using the suggested method. **Table 1** and **Fig.3** Clears the Optimised method chromatographic conditions

Table 1: Optimised method chromatographic conditions

S. No.	Parameter	Chromatographic condition
1	Mobile phase	ACN: Ammonium formate of pH-2.5 adjusted with formic acid (40:60)
2	Column	Waters Symmetry C18 column (150mm x 4.6mm, 3.5µm)
3	Rate of flow	0.2 mL / min
4	The temperature of the column	Ambient temperature
5	The temperature of the sample	Ambient temperature
6	Volume of injection	5 µL
7	Period of run	3 min
8	Retention time	Ritlecitinib Retention time - 1.135 Nilotinib Retention time – 2.044

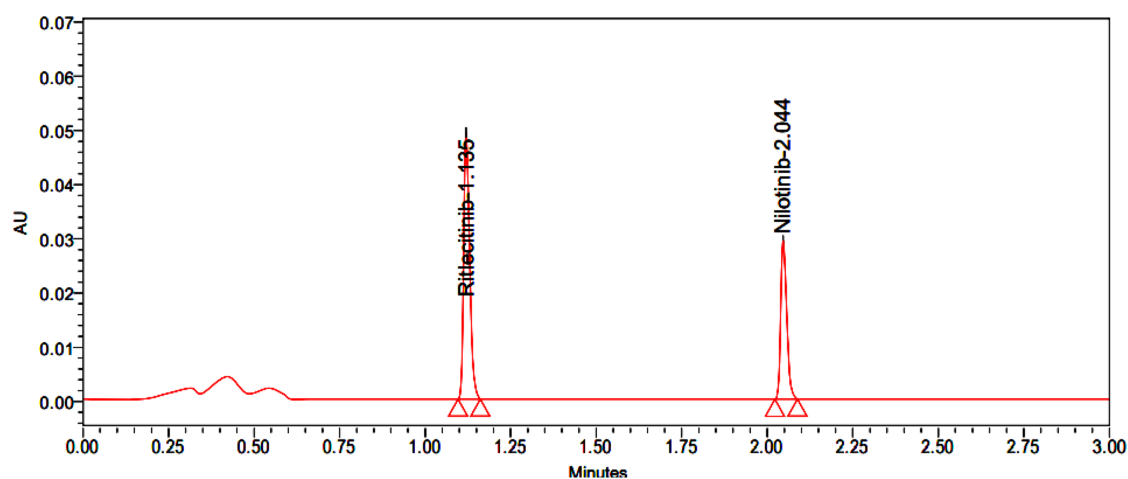

Fig. 3. Chromatogram of standard

4.2 Validation of Bio analytical process

4.2.1 Specificity and Selectivity

Ritlecitinib and IS were used to investigate the rat plasma specimens from six types of rats wastes to examine for blockage from unknown materials. **Fig 1** and **5** indicates the Blank and Blank with IS chromatogram

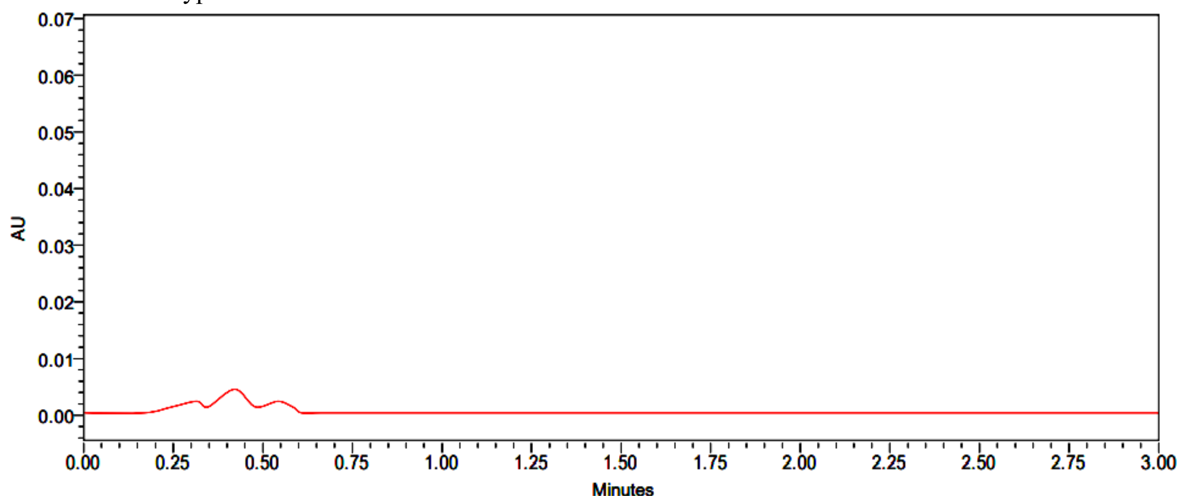

Figure 4: Chromatogram of Blank Rat plasma

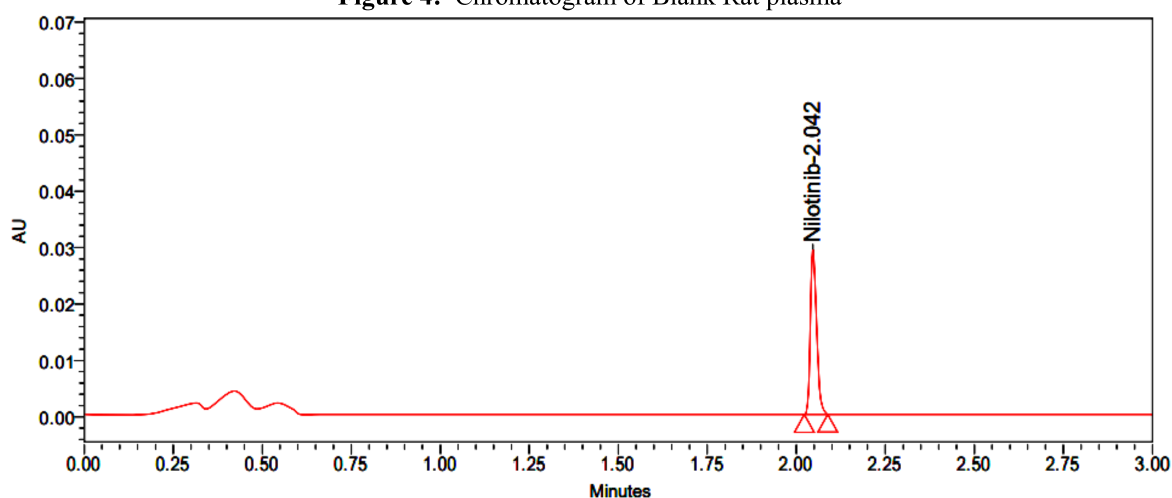

Fig. 5: Blank-IS Chromatogram

4.2.2 System suitability

The System suitability parameter predicts the perfectness and efficacy of the chromatographic status under various analytical parameters. This can be studied by operating the chromatogram for about six replications like MQC-1 to MQC-6. It passed the system suitability. In the proposed approach the CV % of the RT should be less than 2.00 percent. The percent CV of the response ratio should be ≤ 5.00 percent. **Table 2** and **Fig 6** clears the system suitability results.

Table 2: System suitability Results for standard Ritlecitinib

S. No.	Sample Name MQC (5000ng/mL)	Analyte Region(cps)	Analyte RT(min)	ISTD Region (3000ng/mL)	ISTD RT (min)	Region Ratio
1	MQC-1	75133	1.972	46383	1.133	1.6198
2	MQC-2	75286	1.968	46328	1.146	1.6251
3	MQC-3	75230	1.941	46259	1.153	1.6263
4	MQC-4	75348	1.984	46125	1.167	1.6336
5	MQC-5	75118	1.954	46258	1.130	1.6239
6	MQC-6	75324	1.935	46107	1.124	1.6337
Mean		75240	1.959	46243	1.142	1.6271
SD		97.23	0.019	109.29	0.0162	0.0055
%CV		0.13	0.97	0.24	1.42	0.34

Figure 6: Chromatogram of System suitability

4.2.3 Linearity

Over the concentration ranges of 250-7500 ng/mL of Ritlecitinib, drug is prepared and the graphical representation shows linear. Its coefficient was 0.999 on average. The percentage of analyte peak region to internal standard peak region was used to quantify the samples. Plasma concentrations were plotted against peak area ratios shown in the following figure and its linearity report was shown in below **table 3**and **Fig.7**. Inclusion criteria: The linearity regression coefficient should be 0.999.

Table 3: Linearity Outcomes of Ritlecitinib

S. No.	Linearity Level	Final conc. in ng/ml	RES	Area response ratio
1	Linearity-5%	0	0	0.0
2	Linearity-15%	250.00	3778	0.081
3	Linearity-25%	750.00	11405	0.246
4	Linearity-50%	1250.00	18792	0.402
5	Linearity-75%	2500.00	37516	0.806
6	Linearity-100%	3750.00	56324	1.209
7	Linearity-125%	5000.00	75121	1.620
8	Linearity-150%	6250.00	93910	2.022
9	Linearity-200%	7500.00	111234	2.396
Slope			0.000322	
Intercept			0.000692	
R^2 Value			0.99994	

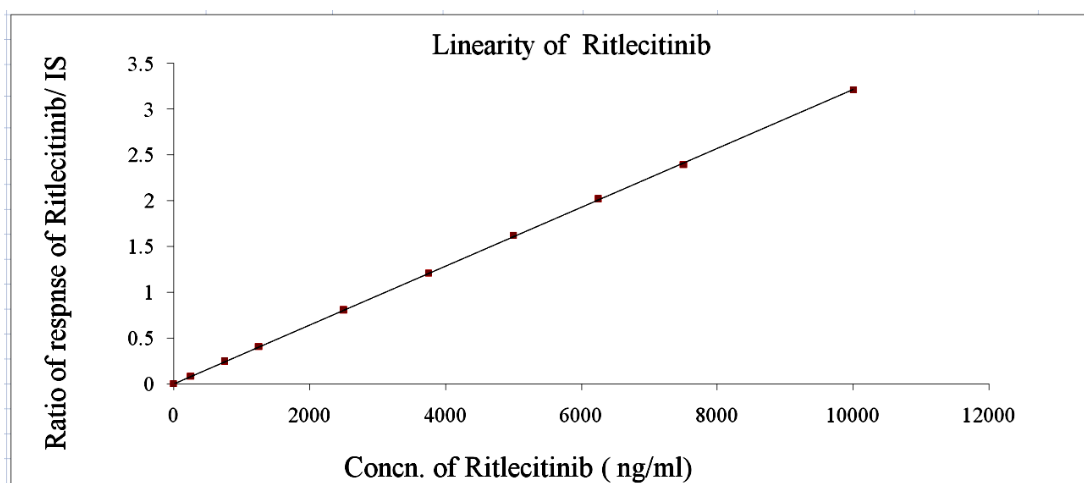

Figure 7: Ritlecitinib Calibration Plot

4.2.4 Sensitivity

It is a significant parameter for the identification of sensitivity of the chromatography to achieve the firmness, authenticity, and lower the uncertainty of the system by operating in six replications with different strengths results in following table and their LLOQ chromatogram is placed in following figure. It passed the sensitivity. Percent accuracy ranges from 80 to 120 for four out of six specimens (**Table 4**). The CV may have an accuracy rate of less than or equal to 20.00%.

Table 4: Sensitivity Results of Ritlecitinib

S. No.	Replicate Number	LLOQ
		Nominal Concentration (ng/ml)
		250
		Peak area
1	1	3572
2	2	3516
3	3	3527
4	4	3589
5	5	3553
6	6	3571
n		6
Mean		3555
SD		28.317
% CV		0.80
% Mean Accuracy		94.50%

4.2.5 Matrix effect

This study comprises the area of a sample after the extraction of plasma with analyte compatibility at equal strengths. The extracted plasma consisting of various ions is a quantified chromatographic approach by identifying rat plasma and ionized ions. Two out of three samples had a percent average exactness of 85-115(**Table 5**). The matrix lot must fulfil at least 80% of the approval criteria. Several biological matrices exhibit LQC of 85 percent and HQC of 115 percent in terms of back-measured concentration accuracy.

Table 5: Matrix effect Outcomes of Ritlecitinib

S. No.	Plasma Lot No.	HQC	LQC
		Nominal Concentration (ng/ml)	
		7500	750
		Area	
1	Lot 1	109585	10981
		109359	11196
		109792	10974
2	Lot 2	109987	10923
		109562	10958
		108366	10964
3	Lot 3	109748	11123
		108221	10847

		109587	10897
4	Lot 4	109745	10951
		109621	10678
		109749	10992
5	Lot 5	109986	10876
		109895	11006
		109893	11084
6	Lot 6	108321	10965
		109487	10943
		109919	11069
n		18	18
Mean		$3.635x10^5$	109490
SD		0.01552	573.514
%CV		0.43	0.52
% Mean Accuracy		99.77%	97.01%
No. of QC Failed		0	0

4.2.6 Precision and Accuracy

Pooling the findings of all QC specimens' tests ensured that the results were accurate and precise. Quality control samples for Ritlecitinib yielded findings of 99.22-99.96 percent accuracy respectively. And the percentage CV of Ritlecitinib was less than 5% for all quality control samples. Exactness and accuracy met or beyond our expectations.LQC, MQC, and HQC samples should have a precision of 15% within and between batches, whereas LLQC samples should have a precision of 20% is acceptable limits in the current approach (**Table 6**).

Table 6: Precision and accuracy Results Ritlecitinib

S. No.	HQC	MQC	LQC	LLQC
	Nominal Concentration (ng/ml)			
	7500	5000	750	250
	Analyte peak area			
1	110394	74123	10866	3569
2	110258	74221	10687	3551
3	112631	74328	10763	3574
4	111243	74458	10924	3523
5	112206	74164	10992	3508
6	110957	74268	10828	3619
n	6	6	6	6
Mean	111282	74260	10843	3557
SD	961.02	121.16	109.77	39.672
% CV	0.86	0.16	1.01	1.12
% Mean Accuracy	98.60%	98.70%	96.07%	94.55%

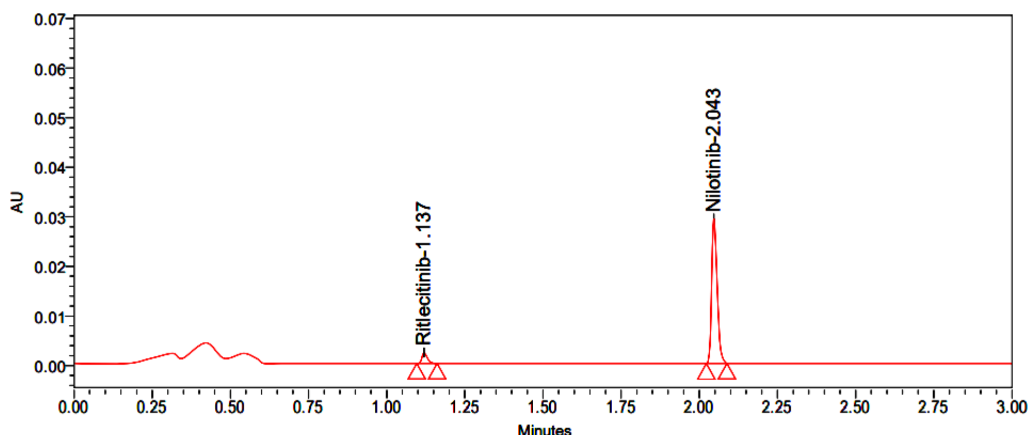

Figure 8: Chromatogram of accuracy and precision

4.2.7 Recovery of analyte

At three different concentrations of lower, medium and higher stages the quality measurements with recoveries of medication& IS were assessed. Recoveries are measured by comparable responses of the sample with neat normal solution responses. The efficacy of extraction is dependent

on how much quantity analyte was used and their results are compared with analytical results. The chromatograms of extracted and un-extracted recoveries of LQC, MQC, and HQC are shown below. In the proposed approach the recovered percent (%RSD) for specific steps like QC and ISTD must be less than or equal to 15%. The net average recovery results are less than or equal to 20 percent and should be found in replicates of QC.**Table 7** clears the outcomes of recovery

Table 7: Recovery results of Ritlecitinib

S. No.	Replicate Number	HQC (7500 ng/ml)		LQC (750 ng/ml)		MQC (5000 ng/ml)	
		Ext Rec	Un Ext	Ext Rec	Un Ext	Ext Rec	Un Ext
1	1	109473	111521	10857	10921	73147	73258
2	2	109574	111248	10816	10879	73051	73169
3	3	109325	110468	10732	10836	73323	73664
4	4	109554	113064	10784	11041	73218	73459
5	5	111963	112446	10665	10792	73154	73287
6	6	109271	110357	10913	11034	73548	73850
n		6	6	6	6	6	6
Mean		109860	111517	10795	10917	73240	73448
SD		1037.396	1074.675	88.54	102.68	175.501	263.91
%CV		0.94	0.96	0.82	0.94	0.24	0.36
%Mean Recovery		97.34%	98.81%	95.65%	96.73%	97.34%	97.62%

4.2.8 Ruggedness on precision accuracy

Precision and accuracy parameters are studied to measure the correctness and, efficacy of the proposed approach for six replications of different strengths. The %CV for Ritlecitinib passed the Ruggedness on precision accuracy based on percentages of SD, CV and accuracy results are acceptable boundaries shown in the below **Table 8** .

Table 8: Ruggedness on precision and accuracy Results of Ritlecitinib

S. No	HQC	LQC	MQC
	Nominal Concentration(ng/ml)		
	7500	750	5000
	Area		
1	109960	10948	73854
2	109876	10823	73365
3	109635	10885	73154
4	108541	10769	73034
5	109794	11147	73551
6	109670	11034	73638
n	6	6	6
Mean	109579	10934	73433
SD	523.155	139.651	308.174
%CV	0.48	1.28	0.42
% Mean Accuracy	97.09%	96.88%	97.60%

4.2.9 Dilution integrity

Diluting this sample with a blank matrix should indicate dilution integrity and analyte matrix fixation across the ULOQC. At ULOQC (10000 ng/mL for Ritlecitinib), dilution integrity was tested (**Table 9**).

Table 9: Dilution integrity Outcomes

S. No.	Analyte	ULOQC conc.	Calculated conc.
1	Ritlecitinib	10000 ng/mL	9999.47 ng/mL

4.3.10 Carryover

A system fault known as a carryover error might affect the sample's measured value. The flow injection technique was used on a UPLC to inject a 5 µL system blank containing acetonitrile and ammonium formate with a pH of 2.5, which had been adjusted with formic acid in a ratio of 40:60. We may assume that the precision and accuracy of the suggested procedure were unaffected by this approach. **Table 10** indicates the carryover results

Table 10: Results of carryover

S. No.	Level	Area of Ritlecitinib
1	Blank	0
2	LLOQC	0.274
3	ULOQC	4.890

4.3.11 Stability

Stability of stock solution was achieved by comparing the

area response of the analyte in the stability samples with the region response of the specimen prepared from the fresh stock solution. Plasma stability experiments were conducted at concentration levels of LQC and HQC using six replicates at each dose. Analyte was considered to be steady if the shift is less than 15 percent according to the guidelines of the USFDA. For 24h, stability of spiked rat plasma samples stored at room temperature (bench top stability) was assessed. Stability of spiked rat plasma deposited in auto sampler 2-8 °C (auto sampler stability) was assessed for 24h. The stability of the auto sampler was led by comparing the extract plasma samples which were injected immediately with the samples which were re-injected at 2-8 °C for 24h after storage in the auto sampler. The durability of the freeze-thaw was achieved by contrasting the durability samples that were frozen at -30°C and three times thawed, with freshly spiked quality control samples. The freeze-thaw stability assessment used six aliquots each of the LQC and HQC concentration ranges. The concentration obtained after 24h was compared with initial concentration for long-term stability assessment. **Table 11** clears the stability results

Table 11: Ritlecitinib Stability findings in rat plasma

S. No.	Stability conditions	Quality Control Points	Average Area ±SD (n=6)	% CV	% Recovery
1	Bench top stability	LQC (750 ng/mL)	10778±59.76	0.55	95.50
		MQC (5000ng/mL)	73022 ± 167.355	0.23	97.05
		HQC (7500ng/mL)	110451± 1128.98	1.02	97.87
2	Freeze-thaw stability (Frozen at -31^{0}C thawed 5 times)	LQC (750 ng/mL)	10912 ± 42.838	0.39	96.69
		MQC (5000ng/mL)	73622 ±228.64	0.31	97.85
		HQC (7500ng/mL)	109851 ± 1390.661	1.27	97.33
3	Short-term stability (7 days at 7^0 C)	LQC (750 ng/mL)	10461±57.032	0.55	92.69
		MQC (5000ng/mL)	71452±243.32	0.34	94.97
		HQC (7500ng/mL)	105784±2934.89	1.77	93.73
4	Long Term Stability (28 days)	LQC (750 ng/mL)	9557±24.493	0.26	84.68
		MQC (5000ng/mL)	64127±222.49	0.35	85.23
		HQC (7500ng/mL)	97179±182.45	0.19	86.11
5	Auto sampler stability at 15°C from 0 to 24h	LQC (750 ng/mL)	10935±130.203	1.19	96.89
		MQC (5000ng/mL)	72989±559.579	0.77	97.01
		HQC (7500ng/mL)	110451±1578.91	1.43	97.87
6	Wet extract stability (12h)	LQC (750 ng/mL)	10918±57.371	0.53	96.74
		MQC (5000ng/mL)	73642±237.68	0.32	97.88
		HQC (7500ng/mL)	110251±878.43	0.80	97.92
7	(18h)	LQC (750 ng/mL)	10884±54.851	0.50	96.44
		MQC (5000ng/mL)	73327±231.68	0.32	97.46
		HQC (7500ng/mL)	109715±205.79	0.19	97.21
8	Dry extract stability (12h)	LQC (750 ng/mL)	10912±45.521	0.42	96.69
		MQC (5000ng/mL)	73537±287.24	0.39	97.74
		HQC (7500ng/mL)	110460±271.74	0.25	97.87
9	(18h)	LQC (750 ng/mL)	10875±34.679	0.32	96.36
		MQC (5000ng/mL)	73470±164.976	0.22	97.65
		HQC (7500ng/mL)	110050±629.63	0.57	97.54

5.0 PHARMACOKINETIC STUDY

Ritlecitinib was isolated from rat plasma using the liquid-liquid extraction technique. In order to do this, 200µl of plasma sample (at the appropriate concentration) was transferred into labelled polypropylene tubes and mixed quickly. Then, 500µl of standard stock and 500 µl of internal standard stock were added, and the mixture was

vortexed for around 10 minutes. Finally, the tubes were centrifuged at 4000rpm at 20°C. I put the sample into auto sampler vials for injection after reconstituting it with 300µl of acetonitrile and 500µl of diluents, which I vortexed briefly. Six separate rats had Ritlecitinib administered into their bodies at various intervals of 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, and 3 hours. Once the samples are ready, they are injected into the chromatographic system and their results are recorded according to the test method. **Table 1** and **Fig.9** clears the kinetic and recovery results

Table 12: Pharmacokinetic parameters of Ritlecitinib

S. No.	Pharmacokinetic Parameters	Ritlecitinib
1	AUC_{0-t}	4936 ng-hr/ml
2	C_{max}	4945.375 ng/ml
3	$AUC_{0-\infty}$	4936 ng-hr/ml
4	t_{max}	0.75 Hrs
5	$T_{1/2}$	2.5 Hrs

Rats were given a single dosage of Ritlecitinib and then samples were taken at various intervals after the treatment: 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, and 3 hours. At each time point, 300 µl of blood was drawn into K2 EDTA vacutainer tubes. Also, in order to rule out any interferences caused by the plasma, a predose sample was taken. We obtained the plasma from the collected samples by centrifuging them, and then we kept them at −70±10 °C. Along with QC samples, plasma samples were spiked with IS and processed at four different concentrations. The WinNonlin software tool (Version 5.2) was used to determine the pharmacokinetic parameters of Ritlecitinib. Incurred sample reanalysis (ISR) confirmed that the research samples were stable. The elimination phase and Cmax, two points in the pharmacokinetic profile, were used to choose two samples from each individual for ISR. The samples were deemed stable if the percentage discrepancy did not exceed ±20%.

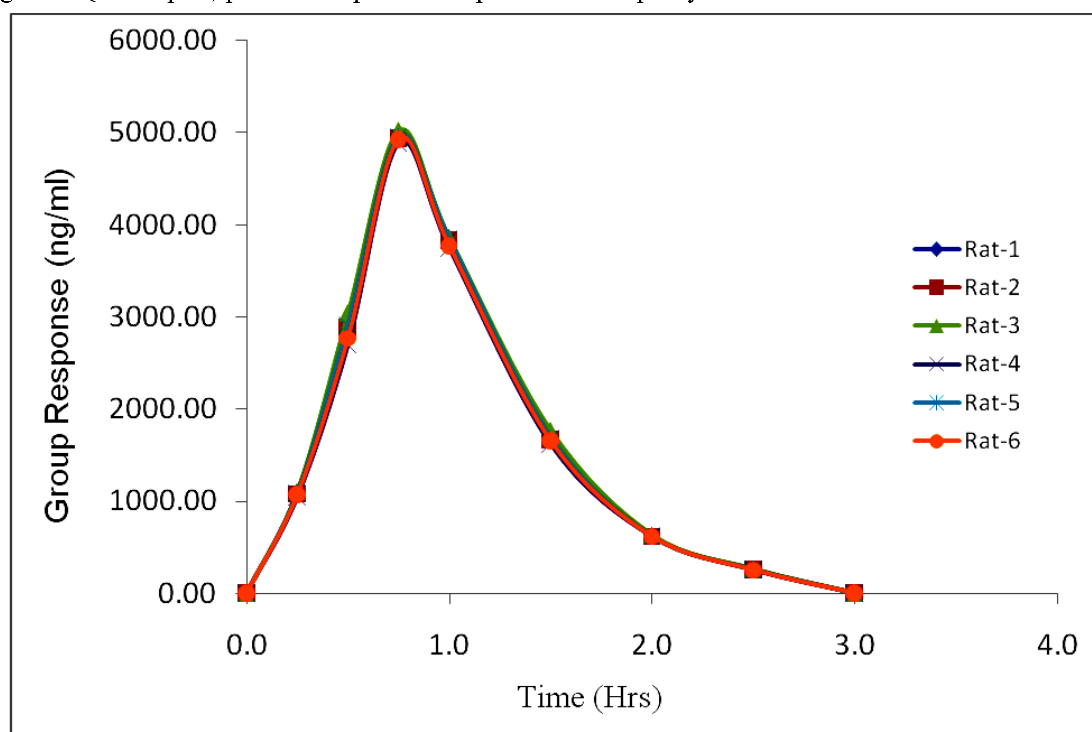

Figure 9: Recovery plot for Ritlecitinib in Rat plasma

6. CONCLUSION

In addition to being robust and reproducible, the created approach has shown exceptional selectivity and linearity. This approach guarantees a reasonably quick analysis by using a straightforward liquid-liquid extraction procedure with little matrix interference and low retention periods of less than three minutes. It is worth mentioning that Ritlecitinib has remarkable stability in rat plasma and achieves recovery rates of about 100% at both low and high concentrations. At the LLOQ, LQC, MQC, and HQC levels, the accuracy (%CV) is less than 15% both within and between batches. In particular, this UPLC-based bioanalytical method offers the first-ever assessment of Ritlecitinib pharmacokinetics and has the ability to considerably simplify the pharmacokinetic evaluation of Ritlecitinib in rats, an essential step in understanding its safety, toxicity, and effectiveness profiles, especially in the field of anticancer research.

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Conflict of Interest
The authors declare that there was no conflict of interest.

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