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Original Article

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

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ABSTRACT

Background: Axitinib was used to treat carcinoma of renal cells and Upadacitinib was used to treat rheumatoid arthritis and Zanubrutinib used as an anti cancer medication for mantle cell lymphoma. In the current application, Zanubrutinib, Upadacitinib and Axitinib and their associated substances will be developed and validated. **Materials and Methods:** The optimised method includes the Zanubrutinib, Upadacitinib and Axitinib gradient elution and associated flow rates with dimensions of 1 mL/min and phenyl column X-bridge (150 x 4.6 mm, 3.5 µ). A mobile phase was employed with 1.2 g of Hexane sulphonic acid of pH-2.5 adjusted with ortho-phosphoric acid (buffer) and acetonitrile. Zanubrutinib, Upadacitinib and Axitinib were separated from their associated substances for a total run time of 60 min. The approach developed has been validated in accordance with the ICH guidelines. **Results:** The test concentration LOD and LOQ were established for both drugs and for their impurities. The diagrams that were drawn were straightforward with an R^2 regression coefficient > 0.999. As part of the method validation, recovery, specificity, linearity, accuracy, robustness was determined and the results were found to be within an acceptable range. **Conclusion:** HPLC was used to validate the method in terms of accuracy, linearity, method precision, accuracy, limit of detection, limit of quantification, robustness, and degradation, and LC-MS/MS was used to characterise the degradation products. The system was validated in all of these aspects.

Keywords: Mobile phase, X-bridge column, Validation, ICH guidelines.

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INTRODUCTION

Zanubrutinib is a treatment medication marketed to adults under the trade name Brukinsa who have already undergone treatment with the Mantle Cell Lymphoma (MCL).[1,2] Efficacy has been evaluated in 86 patients with mantle cell lymphoma (MCLs) who received at least one previous therapy[3] under Phase II, open-label, multicenter, single-arm test BGB-3111-206 (NCT03206970). In phase I/II, open-label, dose escalation, global, multicenter, one-arm test of B cell malignancies was also evaluated for efficacy in BGB-3111-AU-003 (CNT02343120). The primary effect measure in each trial was an Overall Response Rate (ORR). FDG-PET were required for trial BGB-3111-206 with an ORR of 84 percent (95 percent CI: 74, 91), with a full 59 percent (95 percent CI 48, 70) and median 19.5 month response time (95 percent CI: 16.6, not estimable). FDG-PET scans were not required for BGB-3111-AU-003 test and ORR was 84% (95% CI: 67, 95) with a full 22% (95% CI: 9, 400) response rate and a median 18.5 month duration (95 percent CI: 12.6, not estimable).

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When methotrexate isn't working or isn't tolerated by individuals with Janus kinase (JAK),[4] rheumatoid arthritis (RA)[5] inhibitor called upadacitinib, marketed as Rinvoq, is an option (a drug to treat active arthritis). The most frequent adverse effects include infections of the upper respiratory tract,[6] nausea,[7] and cough.[8] (common cold and sinus infections). Janus kinase enzymes are inhibited by Upadacitinib, a cancer drug. Inflammatory processes[9] are set up by these enzymes, and inflammation in joints is controlled by them. Adults with moderate to severe active rheumatoid arthritis who have not responded well to or are intolerant to one or more disease-modifying antirheumatic medications should use Upadacitinib (DMARDs). In addition to[10] or as monotherapy, Upadacitinib can be used. During pregnancy, the medicine is prohibited because of active TB[11] and other severe infections, as well as significant liver impairment (Child–Pugh grade C).[12] Contraindications for the medicine are also included. Upadacitinib should not be used with other Janus kinase inhibitors, biological DMARDs,[13] or immune suppressants[14] such as Azathioprine and Cyclosporine, which are known to depress the immune system. Infections such as pneumonia,[15] cellulitis,[16] TB, and shingles,[17] as well as other herpes disease infections, including life-threatening complications, are among the most serious side effects.

Pfizer is the company that created the small molecule tyrosine kinase inhibitor[18] known as Axitinib, which is sold under the brand name Inlyta. It has been shown to considerably suppress the development of breast cancer[19] in animal (xenograft) models[20] and to induce partial responses in clinical studies with renal cell carcinoma (RCC)[21] and a range of other kinds of tumors.[22] It is hypothesised that its principal mechanism of action is the suppression of vascular endothelial growth factor[23] receptors 1 through 3, c-KIT, and PDGFR.[24] This allows it to block angiogenesis, which is the development of new blood vessels by tumours.[25] It was also hypothesised that it could function by activating autophagy,[26] similar to how certain other tyrosine kinase inhibitors, such as sorafenib do.[27] In addition to this, it has been established[28] that it interacts to the BCR-ABL fusion protein, suppressing particularly the drug-resistant T315I mutant isoform by adopting a different conformation than VEGF does when it does so. More than 20 percent of patients have one or more of the following adverse effects: diarrhoea, hypertension, tiredness, reduced appetite, nausea, dysphonia, hand-foot syndrome,[29] weight loss, vomiting, asthenia, and constipation. These are the most commonly reported side effects. The structural depiction of Zanubrutinib, Upadacitinib, and Axitinib, together with other chemicals that are linked to them, may be Figure 1.

There has been no HPLC technique documented in the scientific literature so far. Thus, we devised a technique for simultaneously determining the concentrations of Zanubrutinib, Upadacitinib, Axitinib, and their related compounds. The HPLC technique established for the *in vitro* determination of the combination of medicines was used.

Analysis of pharmaceutical compounds such as Zanubrutinib, Upadacitinib, Axitinib, and their metabolites is the goal of this research.

MATERIALS AND METHODS

Chemicals

At Merck India Ltd. in Mumbai, India, we were able to purchase acetonitrile with an HPLC mark as well as orthophosphoric acid with an HPLC mark. Spectrum Solution for Pharmacy Research Pvt, Ltd, Hyderabad provided the APIs for Zanubrutinib, Upadacitinib, and Axitinib, as well as their impurities as reference standards.

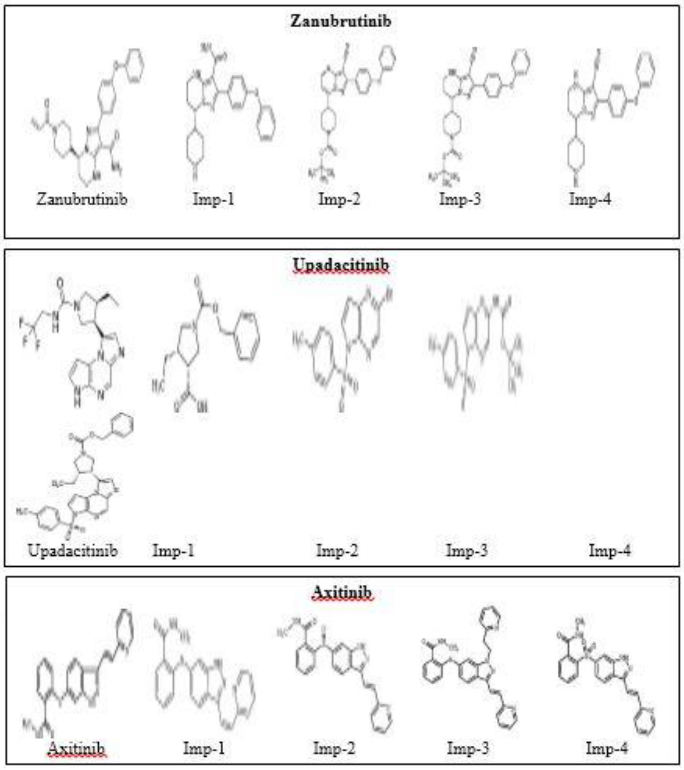


Figure 1: Chemical structures of Zanubrutinib, Upadacitinib, Axitinib and their related substances.

Instrumentation

For this study, the Waters Alliance Liquid Timers (model 2695) monitored with a soft ware of Empower 2.0 management system and a photo diode array detector (model 2998) were used.

Preparation of buffer

After dissolving 1.2 g of hexane sulfonic acid in one litre of HPLC-grade water, the pH of the solution was brought down to 2.5 using orthophosphoric acid. After that, filter the buffer solution using filter paper with a 0.45 micron pore size.

Chromatographic conditions

The HPLC analysis was conducted with a gradient elution mode in the reverse phase HPLC system using a 1 mL/min motive stage of acetonitrile and Hexane sulfonic acid (HSA) and phenyl X-bridge (150 x4.6 mm, 3.5 µ). The gradient programme is Table 1.

Table 1: **Gradient programme.**

Time	Acetonitrile	Buffer (HSA)
0	20	80
15	50	50
30	70	30
50	20	80
60	20	80

Diluents

Mobile phase.

Preparation of the standard solution

The standard solution of Upadacitinib (30 µg/mL), Upadacitinib impurity-1 (1 µg/mL), Upadacitinib impurity-2 (1 µg/mL), Upadacitinib impurity-3 (1 µg/mL), Upadacitinib impurity-4 (1 µg/mL), Zanubrutinib (160 µg/ml), Zanubrutinib impurity-1 (0.5 µg/mL), Zanubrutinib impurity-2 (1 µg/mL), Zanubrutinib impurity-3 (1.5 µg/mL), Zanubrutinib impurity-4 (1 µg/mL), Axitinib (10 µg/ml), Axitinib impurity-1 (0.5 µg/mL), Axitinib impurity-2 (1 µg/mL), Axitinib impurity-3 (1 µg/mL), Axitinib impurity-4 (1 µg/mL) was made by diluting with diluents.

RESULTS

When developing a new method, the main analytical challenge was the separation of active pharmaceutical ingredients. The chromatographic conditions were optimised to ensure good performance.

Method optimization

Diverse phosphate buffer and acetonitrile ratios in mobile phase were tested with isocratic and gradient modes to maximise the chromatographic conditions. However, each test modified the mobile phase composition to improve the resolution and also to reach acceptable retention times. Finally, HSA and acetonitrile with gradient elution were selected because the active pharmacological ingredients and their impurities were more responsive. Various stationary phases such as C_8, C_{18} and amino columns were tested during the optimisation of the method. The top forms of the X-bridge phenyl 150 x 4.6 mm, 3,5 µ with a PDA detector were relatively good from these tests. In order to achieve sufficient sensitivity, the Mobile Flow rate was achieved at 220nm. In the above circumstances, It is obtained a time limit of about 6.311, 14.012 and 24.086 min of Upadacitinib, Zanubrutinib and Axitinib with a tailing factor of 1.02, 1.05 and 1.11. The time for retentions was 2.977, 3.966, 8.257, 12.365 min and for impurities -1, impurities -2, impurities-3 and impurity-4 of Upadacitinib and 13.062, 15.686,21.063,17.390 min of Zanubrutinib, and for Axitinib impurity-1, impurity-2, impurities - 3, or impurities – 4 respectively. 22.921, 26.624, 28.197 and 39.805 min respectively. Retaining times for Upadacitinib's impurity-1, impurity-2 and impurity-4 were 22.921. There were 7,953, 40932 and 70,754 plate theoretical indicators of the column's good results for the Upadacitinib, Zanubrutinib and Axitinib. Less than 2 of the proposed approach indicate that the RSD percentage for six replicate injections has been found to be extremely precise. The method established has been validated according to ICH guidelines.

System suitability

Device fitness parameters such as USP, USP tailings and percent RSD have been assessed. Results of system suitability were shown in Table 2 and the chromatogram was Figure 2.

Specificity

According to the placebo test method, the interference was individually analysed with the test samples and standard solutions. The accompanying Figure demonstrates that the active components could be easily recognised from the blank and its excipients, whereas the placebo had no effect on the primary peak. Because of this, the procedure is rather particular. The blank chromatogram is Figure 3.

Linearity

For Upadacitinib, Zanubrutinib, Axitinib and their related substances, the linearity peak area against different concentrations was evaluated. Test solutions are developed from 10 to 150 percent for the related impurity stock solution substance method at different concentration levels. We have linear connections between peak areas and the relevant pitch concentration in optimal chromatographic conditions. For all components, the correlation coefficients were below the limit.

Table 2: Results of system suitability.

Sl. No	Parameter	Upadacitinib	Zanubrutinib	Axitinib
1	Retention time	6.311	14.012	24.086
2	Plate count	7953	40932	70754
3	Tailing factor	1.02	1.05	1.11
4	Resolution	6.56	3.37	3.33
5	%RSD	0.25	1.79	1.16

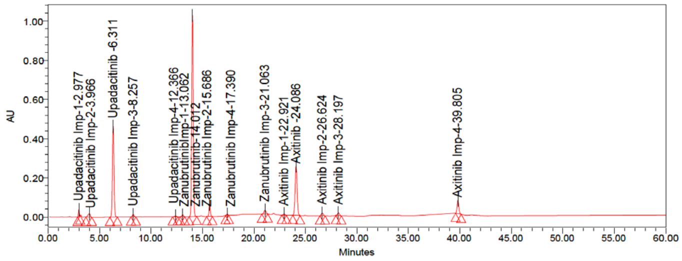


Figure 2: Chromatogram of system suitability.

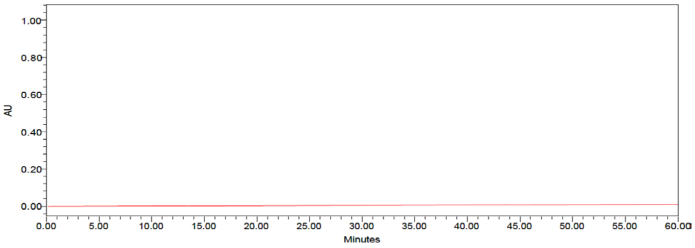


Figure 3: Chromatogram of blank.

Table 3: A, B, C gives the Results of Linearity for Upadacitinib, Zanubrutinib, Axitinib and its impurities.

A

Linearity	Upadacitinib		Imp-1		Imp-2		Imp-3		Imp-4	
	Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area
1	3.00	707566	2.00	58964	1.00	29482	1.00	28415	1.00	14208
2	7.50	1768916	5.00	147410	2.50	73705	2.50	71038	2.50	35519
3	15.00	3537832	10.00	294819	5.00	147410	5.00	142077	5.00	71038
4	22.50	5306748	15.00	442229	7.50	221115	7.50	213115	7.50	106557
5	30.00	7075664	20.00	589639	10.00	294819	10.00	284153	10.00	142077
6	37.50	8844580	25.00	737048	12.50	368524	12.50	355191	12.50	177596
7	45.00	10013496	30.00	854458	15.00	419229	15.00	402230	15.00	201115

B

Linearity	Zanubrutinib		Imp-1		Imp-2		Imp-3		Imp-4	
	Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area
1	16.00	1415133	0.50	56554	1.00	84783	1.50	51487	1.00	82458
2	40.00	3537832	1.25	141387	2.50	211957	3.75	128718	2.50	206145
3	80.00	7075664	2.50	282774	5.00	423915	7.50	257436	5.00	412289
4	120.00	10613496	3.75	424161	7.50	635872	11.25	386154	7.50	618434
5	160.00	14151328	5.00	565548	10.00	847829	15.00	514872	10.00	824578
6	200.00	17689160	6.25	706935	12.50	1059786	18.75	643590	12.50	1030723
7	240.00	20226992	7.50	798322	15.00	1231744	22.50	729308	15.00	1166867

C

Linearity	Axitinib		Imp-1		Imp-2		Imp-3		Imp-4	
	Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area
1	1.00	145786	0.50	28745	1.00	53699	1.00	55479	1.00	74851
2	2.50	364466	1.25	71863	2.50	134247	2.50	138697	2.50	187128
3	5.00	728932	2.50	143725	5.00	268494	5.00	277395	5.00	374256
4	7.50	1093397	3.75	215588	7.50	402740	7.50	416092	7.50	561384
5	10.00	1457863	5.00	287451	10.00	536987	10.00	554789	10.00	748512
6	12.50	1822329	6.25	359313	12.50	671234	12.50	693486	12.50	935640
7	15.00	2066795	7.50	408177	15.00	760481	15.00	788184	15.00	1062768

The linearity results were shown in Table 3 and the calibration plots were shown in Figure 4.

Accuracy

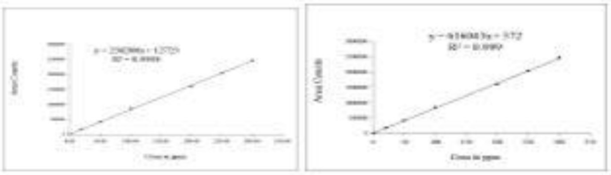
Accuracy in 3 different types of concentrations of 50, 100 and 150 per cent each at a given limit was carried out through analysis of active pharmaceuticals solution spotted with known amounts from all impurities. It was determined that the recovery percentages were within acceptable limits for all contaminants. Accuracy results were in Table 4.

Precision

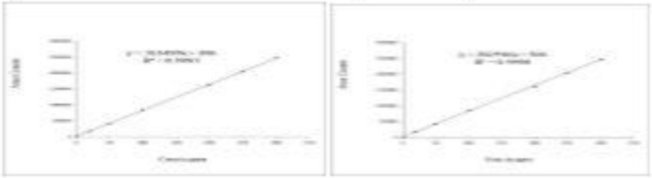
A method's precision is determined by how close a series of measurements from several homogenous mixture samples are to one another. Six individual injection determinations of Upadacitinib, Zanubrutinib, Axitinib and related substances were injected to determine the exactness of the process of associated substances. The percent RSD of each impurity has been determined and the results showed that under the specified experimental conditions the technology is accurate. Intraday precision results were in Table 5 and Figure 5 gives the sample chromatogram.

Ruggedness

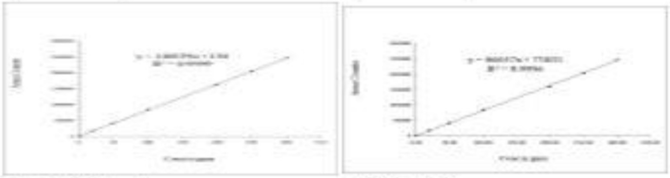
In all, six duplicates of the solution standard were evaluated by six different analyzers and on distinct days, using different tools. The drug responses were measured for a medium percentage of RSD



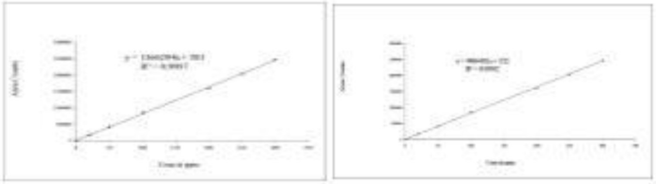
Upadacitinib Upadacitinib imp-1



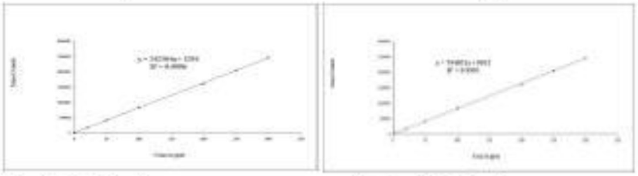
Upadacitinib imp-2 Upadacitinib imp-3



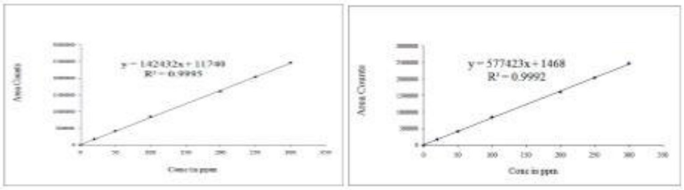
Upadacitinib imp-4 Zanubrutinib



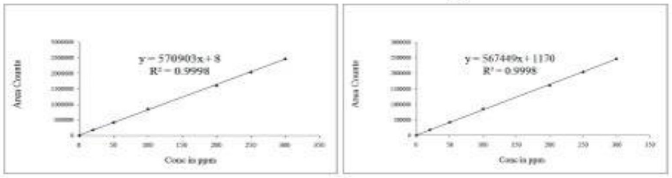
Zanubrutinib imp-1 Zanubrutinib imp-2



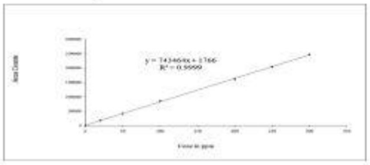
Zanubrutinib imp-3 Zanubrutinib imp-4



Axitinib Axitinib imp-1



Axitinib imp-2 Axitinib imp-3



Axitinib imp-4

Figure 4: Calibration plots of Upadacitinib, Zanubrutinib, Axitinib and their impurities.

Table 4: A, B, C gives the results of Accuracy.

A					
Amount spiked	% Recovery				
	Upadacitinib	Imp-1	Imp-2	Imp-3	Imp-4
50%	100.0	100.7	100.2	99.9	98.7
100%	99.7	99.8	98.5	99.8	100.2
150%	101.6	100.4	100.1	99.4	100.3

B					
Amount spiked	% Recovery				
	Zanubrutinib	Imp-1	Imp-2	Imp-3	Imp-4
50%	101.6	101.1	100.4	99.0	100.6
100%	101.9	99.8	100.0	99.9	99.9
150%	100.4	99.4	99.9	99.1	100.4

C					
Amount spiked	% Recovery				
	Axitinib	Imp-1	Imp-2	Imp-3	Imp-4
50%	99.2	99.3	99.2	100.0	100.8
100%	98.7	100.3	100.0	99.9	100.0
150%	98.8	100.3	100.0	98.6	99.7

Table 5: A, B, C gives the Intraday precision results.

A			
Sample number	Upadacitinib % Related substances		
	Known impurities	Total impurities	% Purity (100-Known impurities)
1	5.17	5.17	94.83
2	5.11	5.11	94.89
3	5.10	5.10	94.90
4	5.19	5.19	94.81
5	5.15	5.15	94.85
6	5.24	5.24	94.76
Average	5.16	5.16	94.84
% RSD	1.01	1.01	0.05

B			
Sample number	Zanubrutinib % Related substances		
	Known impurities	Total impurities	% Purity (100-Known impurities)
1	5.62	5.62	94.38
2	5.65	5.65	94.35
3	5.67	5.67	94.33
4	5.66	5.66	94.34
5	5.59	5.59	94.41
6	5.64	5.64	94.36
Average	5.64	5.64	94.36
% RSD	0.52	0.52	0.03

continued...

Table 5: Cont'd.

C			
Sample number	Axitinib % Related substances		
	Known impurities	Total impurities	% Purity (100-Known impurities)
1	5.55	5.55	94.45
2	5.42	5.42	94.58
3	5.43	5.43	94.58
4	5.48	5.48	94.52
5	5.40	5.40	94.60
6	5.49	5.49	94.51
Average	5.46	5.46	94.54
% RSD	1.02	1.02	0.06

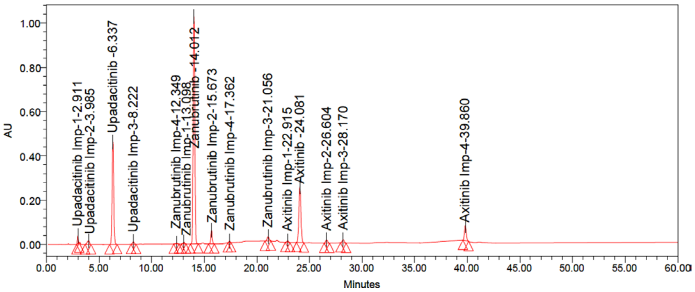


Figure 5: Chromatogram of sample.

Table 6: A, B, C gives the Inter-day outcomes.

A			
Sample number	Upadacitinib % Related substances		
	Known impurities	Total impurities	% Purity (100-Known impurities)
1	5.37	5.37	94.63
2	5.34	5.34	94.66
3	5.42	5.42	94.58
4	5.33	5.33	94.67
5	5.39	5.39	94.61
6	5.35	5.35	94.65
Average	5.37	5.37	94.63
% RSD	0.63	0.63	0.04

B			
Sample number	Zanubrutinib % Related substances		
	Known impurities	Total impurities	% Purity (100-Known impurities)
1	5.72	5.72	94.28
2	5.76	5.76	94.24
3	5.74	5.74	94.26
4	5.73	5.73	94.27
5	5.78	5.78	94.22
6	5.75	5.75	94.35
Average	5.75	5.75	94.27
% RSD	0.38	0.38	0.05

C			
Sample number	Axitinib % Related substances		
	Known impurities	Total impurities	% Purity (100-Known impurities)
1	5.52	5.52	94.48
2	5.58	5.58	94.42
3	5.51	5.51	94.49
4	5.59	5.59	94.41
5	5.53	5.53	94.47
6	5.50	5.50	94.50
Average	5.54	5.54	94.46
% RSD	0.68	0.68	0.04

Table 7: Results of LOD and LOQ.

Component	LOD		LOQ	
	Concentration (µg/mL)	S/N	Concentration (µg/mL)	S/N
Upadacitinib	0.5	7	5	26
Imp-1	0.02	3	0.2	22
Imp-2	0.01	5	0.1	25
Imp-3	0.01	3	0.1	21
Imp-4	0.01	4	0.1	22
Zanubrutinib	1	8	10	28
Imp-1	0.005	5	0.05	23
Imp-2	0.01	6	0.1	24
Imp-3	0.015	5	0.15	21
Imp-4	0.01	3	0.1	22
Axitinib	0.125	7	1.25	21
Imp-1	0.005	3	0.05	25
Imp-2	0.01	3	0.1	22
Imp-3	0.01	3	0.1	25
Imp-4	0.01	5	0.1	22

values. The results are given in the table below. Table 6 gives the inter-day precision results.

LOD and LOQ

LOD and LOQ of the compounds were continuously injected into the lower compounds. LOD and LOQ results were given in Table 7 and the Figure 6 shows the LOD and LOQ chromatograms.

Robustness

All of these diverse circumstances, such as fluid rate and organic mobile phase %, have been created to evaluate the durability of the constructed system. Retention duration, count, and tailing were not substantially affected by contaminants, and neither were active medicinal components. This was therefore a robust method. Table 8 gives the robustness data.

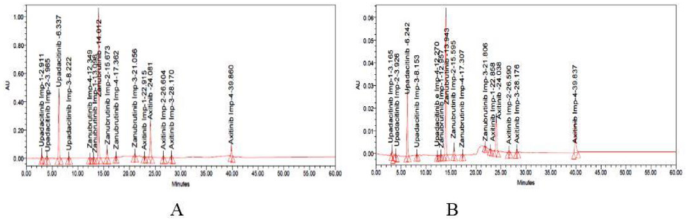


Figure 6: Chromatogram of (A) LOD and (B) LOQ.

Table 8: Robustness data.

Parameter name	% RSD		
	Upadacitinib	Zanubrutinib	Axitinib
FM (0.8 ml/min)	1.22	0.76	0.63
FP (1.2 ml/min)	0.84	0.44	0.35
OM (-10%)	0.67	0.59	0.88
OP (+10%)	0.23	0.47	0.51

Table 9: Forced degradation results.

Stress condition	% Purity		
	Upadacitinib	Zanubrutinib	Axitinib
Acid degradation	83.5	84.9	82.9
Alkali degradation	82.8	84.6	82.2
Peroxide degradation	84.7	83.7	83.4
Reduction degradation	82.1	82.8	82.6
Thermal degradation	81.6	82.5	81.7
Photolytic degradation	81.2	80.6	80.9
Hydrolysis degradation	80.9	81.4	80.3

Degradation studies

In order to achieve just a partial breakdown of the medication, the normal preparations of Upadacitinib, Zanubrutinib, and Axitinib were treated to numerous circumstances that accelerated their degradation. Studies of forced degradation have been carried out in order to determine whether or not the procedure is suitable for the products of degradation. Using LC-MS, the generated degradation products were analysed in order to characterise them. Degradation data was in Table 9 and the mass spectr as were Figure 7.

Acid degradation

1 millilitre of standard stock solution is transferred to a volumetric flask containing 10 millilitres; then, 1 millilitre of 1N HCl is added, and the mixture is let to stand for 15 min. After waiting 15 min, add one millilitre of 1N sodium hydroxide and make it up to the mark using diluents. DP1 and DP2 are the two degradation products that were produced as a result of acid degradation.

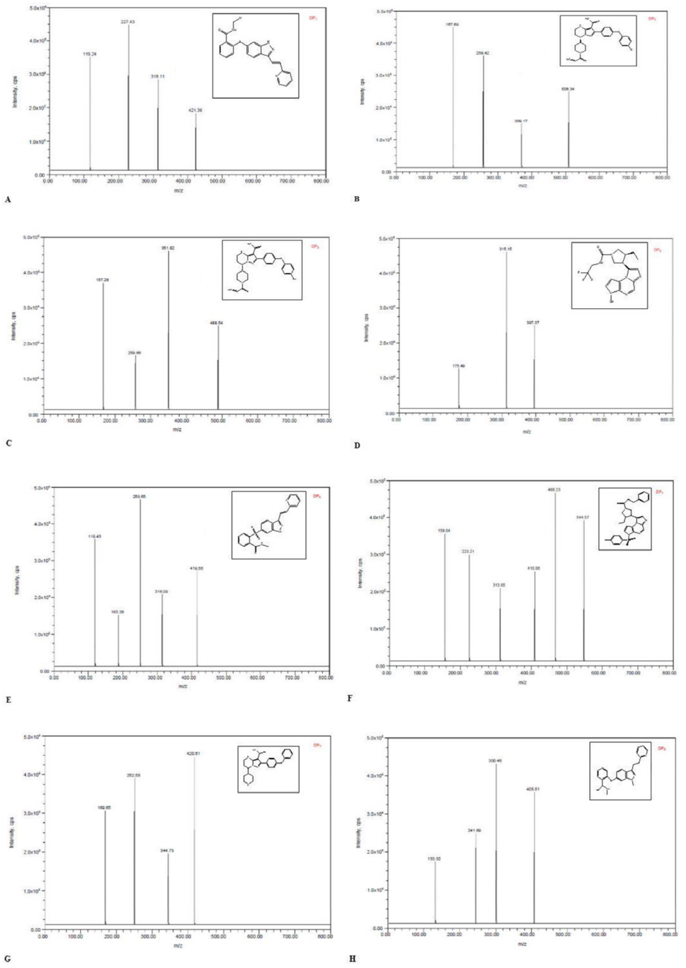


Figure 7: Mass spectras of degradation products formed in various degradation conditions.

Alkali degradation

After adding 1 ml of 1N sodium hydroxide and waiting 15 min, 1 ml of standard stock solution was placed in a volumetric pipette that had a capacity of 10 ml. After 15 min, add 1 ml of 1N HCl and bring the total volume up to the mark using diluents. DP3 and DP4 are the names of the two degradation products that were produced as a result of alkali degradation.

Peroxide degradation

In a volumetric flask with a capacity of 10 millilitres, one millilitre of standard stock solution was transferred, 0.3 millilitres of hydrogen peroxide at a concentration of 30 percent was added, and the volume was brought up to the appropriate level using diluents. The breakdown of peroxide results in the formation of two different degradation products, designated DP5 and DP6, respectively.

Reduction degradation

After transferring 1 ml of standard stock solution into a 10 ml volumetric flask, 1 ml of a solution containing 30% sodium bi sulphate was added, and the volume was brought up to the appropriate level using diluents. Through the process of reduction degradation, just one degradation product, designated as DP7, was produced.

Thermal degradation

In order to establish the standard solution, it was baked in an oven at 105 degrees for six hours. The produced solution was then put through HPLC for analysis. The heat degradation process did not result in the formation of any degradation products.

Hydrolysis degradation

After transferring 1 ml of standard stock to a volumetric flask containing 10 ml, 1 ml of water was added, and the solution was diluted up to the mark. The hydrolysis method of degradation did not result in the formation of any degradation products.

Photolytic degradation

After transferring 1 ml of standard stock to a volumetric flask containing 10 ml, 1 ml of water was added, and the solution was diluted up to the mark. This solution was left out in the sun for three hours, and then the results of the exposed standard were evaluated. The process of photo degradation resulted in the formation of only one DP8 degradation product.

Stability

At room temperature and at 2-8°C, the standard solution was kept up to 24 hr. It was then necessary to run the system for 24 hr with this solution and determine the deviation %. The results were consistent for up to 24 hr, and no significant changes were seen. Table 10 displays the stability data.

DISCUSSION

The mobile phase with composition of Hexane sulphonic acid (pH-2.5 adjusted with Ortho phosphoric acid) and Acetonitrile with gradient elution proven effective and quick separation of chosen pharmacological compounds, its associated impurities and their degradants generated during forced degradation research. Method's system adequacy was assessed by analysing the characteristics such as, retention time, area, theoretical plates, resolution and tailing factor. The present experiment was done on X-bridge phenyl (150mm × 4.6mm, Agilent 5μm) column at ambient temperature which demonstrated greater repeatability. The result of system suitability parameters were determined to be within acceptable stated ranges as per recommendations of International Council on Harmonization (ICH) guideline. The straight baseline was discovered in the blank chromatogram of mobile phase and both medicines, its associated impurities were efficiently resolved in active ingredient form. The calculated detection and quantification limit values revealed that approach was adequately sensitive for the detection of drug in the presence of their degradants. Also, % recoveries of the medicines were reported to be within permissible levels. Method's Specificity was tested by injecting blank, standard, sample and undergoing forced degradation. The statistical data gathered during robustness research show that the approach which is created is resilient. In the degradation investigation, the produced degradants were characterised by utilising LC-MS/MS.

Table 10: Stability results.

Stability	Upadacitinib		Zanubrutinib		Axitinib	
	Purity	% Deviation	Purity	% Deviation	Purity	% Deviation
Initial	94.27	0.00	94.35	0.00	94.52	0.00
6 hr	94.05	-0.02	94.12	0.36	94.41	0.19
12 hr	93.98	1.02	93.74	1.14	94.29	0.24
18 hr	93.42	1.15	93.52	1.32	93.86	1.01
24 hr	93.24	1.24	93.13	1.48	93.35	1.27

CONCLUSION

It is proposed that the stability that is straightforward, selective, verified, and well stated. It demonstrates that Upadacitinib, Zanubrutinib and Axitinib and the chromatographic impurities of their gradients were determined in a quantitative RP-HPLC method. All degradation products formed under stress conditions and the associated uncleanness of the active ingredients in pharmaceuticals were clearly separated and peaks were well resolved, with a suitable retention time that indicates that the method proposed is quick, simple, feasible and inexpensive in an RS situation. Stability studies and regular examination of manufacturing samples might benefit from this technology since it can be utilised in both cases.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

RP-HPLC: Reverse phase high performance liquid chromatography; **LC-MS:** Liquid chromatographic mass spectrometry; **LOD:** Limit of detection; **LOQ:** Limit of quantification; **ICH:** International Conference on Harmonization; **PDA:** Photo diode array detector; **RSD:** Relative standard deviation; **HCl:** Hydro chloric acid; **NaOH:** Sodium hydroxide; **DP:** Degradation product; **RS:** Related substances.

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