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VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF AXITINIB AND AVELUMAB BY USING ANALYTICAL QUALITY BY DESIGN (AQbD) METHOD

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Keywords:

RP-HPLC, Analytical quality by design (AQbD), Central Composite Design, Axitinib and Avelumab

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ABSTRACT: The quantitative measurement of Axitinib and Avelumab has been created using a simple, quick, precise, sensitive, and reproducible reverse-phasehigh-performance liquid chromatography (RP-HPLC) method. It is more difficult to analyse varying amounts of pharmaceutical active medicinal ingredients in dose forms without any interferences. Therefore, the objective of the current work is to estimate Axitinib and Avelumab simultaneously by adopting an Analytical Quality by Design (AQbD) arotatable central composite-based technique using RP-HPLC-based method development and validation. Axitinib and Avelumab were separated by chromatography using a Hyperclone 5µ BDS C18 130A (150 x 4.6 mm, 5µ) column and a mobile phase made up of Acetonitrile: 0.1% TEA pH-2.5/OPA in a ratio of 45:55 v/v. The flow rate was 1.2 ml/min, and a Photodiode Array Detector operating at room temperature was used to detect absorption at 219 nm. ICH criteria have been used to validate the offered techniques' linearity, accuracy, precision, and other attributes. The degradation study's findings showed that the medications deteriorated in high-stress situations. The chemical and pharmaceutical sectors might easily implement this unique AQbD-based analytical technique for routine analysis without any regulatory constraints.

INTRODUCTION: Clear-cell renal-cell carcinoma, the most common kind of renal cancer, is characterised by genetic defects that cause excessive production of vascular endothelial growth factor (VEGF), a crucial regulator of angiogenesis. Even though sunitinib is a first-line treatment option that is considered to be the standard of care for patients with advanced renal cell carcinoma, many of these patients have an innate resistance to antiangiogenic medications or have a progressing disease.

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Avelumab, an anti-programmed death ligand 1 (PD-L1) antibody, is one type of immune checkpoint inhibitor. As first- and second-line treatments for patients with a variety of tumour types, including advanced renal-cell carcinoma, these medications have been found to have acceptable safety and long-lasting anticancer activity [1].

VEGF receptor (VEGFR) inhibitors have immunomodulatory effects in addition to antiangiogenic effects. These effects include increased immune cell infiltration of tumours and decreased immunosuppressive activity of myeloid-derived suppressor cells. We predicted that the complimentary modes of action of an immune checkpoint inhibitor and a VEGF-targeted antiangiogenic treatment may increase the therapeutic benefits. Advanced renal cell carcinoma

is a condition for which axitinib, a highly selective VEGFR inhibitor, is approved for treatment [2]. Since, the drug combination was being tested in clinical studies for the treatment of renal cancer, relatively few analytically fundamental approaches were reported. However, up until this point, no reports have been made on the estimation of axitinib and avelumab stability using the quality-by-design method. This research's initial report will include a thorough analysis of techniques variables, a complete profile of the targeted medicine, and risk assessment considerations. The QbD methodology, which provides the precise interaction of several variables at a time on method response, completely overcame the drawbacks of the classic one-factor altering method. To estimate Axitinib and Avelumab via the QbD technique, a simple, sensitive, precise, robust selective stability indicating RP-HPLC method was devised.

MATERIALS AND METHODS:

Drugs, Chemicals, Solvents, Instruments and Software: Axitinib and Avelumabare generously gifted from the Shree Icons laboratories, in Vijayawada, India. HPLC grade Tri Ethyl Amine (TEA) was purchased from Thermo Fisher Scientific (Maharashtra, India). HPLC grade acetonitrile and orthophosphoric acid (OPA) from Rankem fine chemicals limited (NewDelhi, India).

The High-performance liquid chromatographic (HPLC) system utilized for the whole analysis was Waters e 2695HPLC (Wilmslow, England), united with a double solvent manager with a photodiode array detector (PDA) along with an auto sampler. Unichrome ultrasonic baths have been used to solubilize and degas the sample and solvents. The pH of the mobile phase was adjusted with an Eutech Digital pH Meter (Maharashtra, India). Waters HPLC system unified with Empower 2.0 software for data management. AQbD was developed using Design-Expert® trail version 13 (Stat-Ease Inc., Minneapolis-USA).

Solutions Preparations:

Preparation of Standard: Accurately weigh and transfer 5 mg of Axitinib, and 20 mg of Avelumab working standard into a 10 ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 1 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent. (50ppm of Axitinib, 200ppm of Avelumab)

Preparation of Sample: Accurately weigh and transfer 83.4 mg of Axitinib tablet powder and 1ml of Avelumab sample into a 10mL clean dry volumetric flask add diluent and sonicate it up to 30 mins to dissolve, and centrifuge for 30min. to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through a 0.45-micron Injection filter (Stock solution). Further pipette 1 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluents. (50ppm of Axitinib, 200ppm a of Avelumab).

Final Optimized Chromatographic Conditions in RP-HPLC using AQbD [3, 4]: The separation and determination of two drugs have been achieved with the help of rotatable central composite-based AQbD using Hyperclone 5µ BDS C18 130A (150x4.6 mm, 5 µ) column. The mobile phase comprises HPLC grade acetonitrile: 0.1% TEA (pH adjusted to 2.5 using orthophosphoric acid) in a ratio of 45: 55 v/v. The optimized chromatographic conditions were validated according to the ICH Q2R1 guidelines for specificity, linearity, accuracy, precision, precision, LOD & LOQ and ICH Q2B for degradation studies.

System Suitability [5]: The tailing factor for the peaks due to Axitinib and Avelumab in the Standard solution should not be more than 2.0. Theoretical plates for the Axitinib and Avelumab peaks in the Standard solution should not be less than 2000. Resolution for the Axitinib and Avelumab peaks in standard solution should not be less than 2.

Assay: Inject 10 µL of the standard, sample into the chromatographic system and measure the areas for Axitinib and Avelumab peaks and calculate the percentage.

Specificity: The specificity of an analytical method is the ability to measure specifically the analyte of interest without interference from blank and known impurities. For this purpose, blank chromatogram, standard chromatogram and sample chromatogram were recorded. The chromatogram of blank shows

no response at the retention times of drugs which confirms the reaction of drugs was specific [6].

Linearity:

Preparation of Stock Solution: Accurately weigh and transfer 5mg of Axitinib, and 20mg of Avelumab working standard into a 10ml clean dry volumetric flask add Diluent and sonicate to dissolve it entirely and make volume up to the mark with the same solvent (Stock solution). Prepare 6 levels of samples with varying concentrations *viz.*, 12.5, 25, 37.5, 50, 62.5, and 75 ppm of Axitinib and 50, 100, 150, 200, 250, 300 ppm of Avelumab respectively.

Procedure: Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on the X-axis concentration and Y-axis Peak area) and calculate the correlation coefficient [7].

Accuracy [8]: Concerning target Assay concentration were prepared i.e., 50, 100 & 150 % solutions of Axitinib and Avelumab and determined.

Procedure: Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% keys.

Acceptance Criteria: The percentage recovery for each level should be between 98.0 to 102.0%.

Precision [9]: Precision is the degree of repeatability of an analytical method under normal operation conditions. Precision is of 3 types

1. System precision

2. Method precision (Repeatability)

3. Intermediate precision (a. Intraday precision, b. Inter day precision)

System precision is checked by using standard chemical substances to ensure that the analytical system is working properly. In this peak area and % of drug of six determinations is measured and % RSD should be calculated. In method precision, a homogenous sample of a single batch should be analyzed 6 times. This indicates whether a method is giving constant results for a single batch. In this analyze the sample six times and calculate the % RSD. The precision of the instrument was checked by repeatedly injecting (n=6) solutions of 50ppm of Axitinib and 200ppm of Avelumab).

Acceptance Criteria: The % RSD for the absorbance of six replicate injection results should not be more than 2%.

Degradation Studies:

Preparation of Stock: Accurately weigh and transfer 83.4 mg of Axitinib and 1 ml of Avelumab sample into a 10 ml clean dry volumetric flask add Diluent and sonicate to dissolve it entirely and make volume up to the mark with the same solvent. (Stock solution)

Acid Degradation: Pipette 1 ml of the aforementioned solution was added to a 10 ml vacuum flask, followed by 1 ml of 1N HCl. The vacuum flask was then maintained at 60°C for 1 hour before being neutralised with 1 N NaOH and diluted to 10ml with diluent. Filter the solution using 0.22-micron syringe filters and transfer it to bottles.

Alkali Degradation: Pipette 1 ml of the above solution into a 10 ml volumetric flask and add 1ml of 1N NaOH was added. Then, the volumetric flask was kept at 60ºC for 1 hour and then neutralized with 1N HCl and made up to 10ml with diluent. Filter the solution with 0.22-micron syringe filters and place it in vials.

Thermal Degradation: Axitinib and Avelumab sample was taken in a Petri dish and kept in a Hot air oven at 105°C for 3 hours. Then the sample was taken and diluted with diluents injected into HPLC and analysed.

Peroxide Degradation: Pipette 1 ml above stock solution was added to a 10 ml vacuum flask, 1 ml of 3 per cent w/v hydrogen peroxide was added to the flask and the volume was built up to the mark using diluent. The vacuum flask was then maintained at 60^{o}C for 1 hour. After that, the vacuum flask was left at room temperature for 15 minutes. Filter the solution using 0.22-micron syringe filters and transfer it to bottles.

Reduction Degradation: Pipette 1ml of the above-stock solution was added to a 10ml vacuum flask, 1ml of 10% Sodium bisulphate was added to a flask and the volume was built up to the required

volume with diluent. The vacuum flask was then maintained at 60°C for 1 hour. After that, the vacuum flask was left at room temperature for 15 minutes. Filter the solution using 0.22-micron syringe filters and transfer it to bottles.

Photolytic Degradation: Axitinib and Avelumab sample was placed in a Photo stability chamber for 3 hours. Then the sample was taken and diluted with diluents injected into HPLC and analysed.

Hydrolysis Degradation: Pipette 1ml of above-stock solution was added to a 10ml vacuum flask, 1ml of HPLC grade water was added to a flask and the volume was built up to the required volume with diluent. The vacuum flask was then maintained at 60°C for 1 hour. After that, the vacuum flask was left at room temperature for 15 minutes. Filter the solution using 0.22-micron syringe filters and transfer it to bottles.

RESULTS AND DISCUSSION:

Method Development: The mobile phase used for chromatographic separation was acetonitrile: 0.1% TEA (pH adjusted to 2.5 using orthophosphoric acid) in a ratio of 45: 55 v/v at a flow rate of 1 mL/min. The column temperature was kept at 25°C, and 219 nm detection was used. Axitinib and Avelumab'sretention times were 2.141 minutes and 3.832 minutes, respectively **Fig. 1**. The optimized chromatographic conditions were shown in **Table 1.**

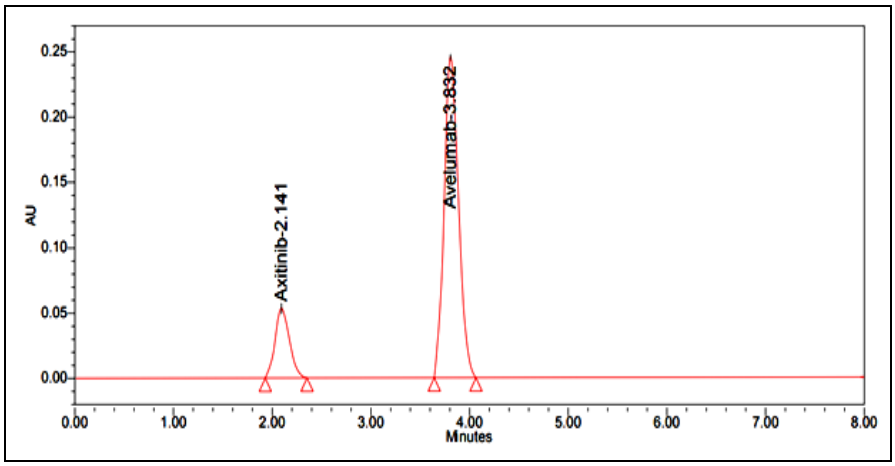

FIG. 1: OPTIMIZED CHROMATOGRAM OF AXITINIB AND AVELUMAB

TABLE 1: OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Parameters	Observation
Instrument used	Waters HPLC with an autosampler and PDA detector
Injection volume	10µl
Mobile Phase	Acetonitrile: 0.1% TEA pH-2.5/OPA (45:55)
Column	Hyperclone 5µ BDS C18 130A (150x4.6 mm, 5 µ)
Detection Wave Length	219nm
Flow Rate	1.2- mL/min
Runtime	8min
Temperature	Ambient(25° C)
Mode of separation	Isocratic mode

Design of Experiment using AQbD: Mobile phase ratio, mobile phase pH, and column temperature were shown to be the analytical characteristics that have an impact on the performance of the method based on the chosen chromatographic settings. The method responses selected were the number of theoretical plates and resolution between degradant peak 1 and degradant peak 2 which likely co-elute and lead to method failure often. 2-factor 3-level factorial designs were preferred in the response surface method. The selected method responses and their levels are given in **Table 1. Table 2** lists the summary of solutions used and selected. Thirteen chromatogram runs were carried out using the central composite design in Design Expert software **Table 3.**

TABLE 2: SUMMARY OF SOLUTIONS USED AND SELECTED

Number	Organic phase	Flow rate	Peak one theoretical plates	Resolution	Desirability	
1	45.000	1.200	3268.200	5.899	0.826	Selected
2	45.000	1.198	3266.146	5.902	0.825	
3	44.951	1.200	3257.568	5.898	0.819	
4	44.889	1.200	3244.348	5.896	0.812	
5	45.000	1.181	3242.485	5.937	0.810	
6	45.000	1.173	3232.087	5.952	0.804	
7	45.000	1.164	3220.367*	5.968	0.797	
8	45.000	1.136	3185.427	6.011	0.777	
9	45.000	0.890	2944.417	6.028	0.634	

TABLE 3: CENTRAL COMPOSITE DESIGN FOR SCREENING OF METHOD PARAMETERS

Std	Run	Factor 1 A:Organic Phase	Factor 2 B:Flow rate	Response 1 Peak one theoretical plates	Response 2 Resolution
2	1	45.0	0.80	2876	5.59
7	2	40.0	0.72	2085	5.59
11	3	40.0	1.00	2216	5.94
9	4	40.0	1.00	2224	5.88
4	5	45.0	1.20	3198	5.88
13	6	40.0	1.00	2239	5.93
10	7	40.0	1.00	2299	5.63
5	8	32.9	1.00	1876	4.95
1	9	35.0	0.80	1945	4.95
12	10	40.0	1.00	2238	5.83
3	11	35.0	1.20	2067	5.33
8	12	40.0	1.28	2566	5.33
6	13	47.1	1.00	3562	6.32

Statistical Analysis of Method Response 1 – Theoretical Plates: ANOVA for method response 1: The Design Expert programme provided the analysis of variance (ANOVA) regression parameters for the projected response surface quadratic model for the number of theoretical plates **Table 4**. The model is important given its Model F-value of 175.22. A "Model F-value" this large could only occur owing to noise with a 0.0001% chance. When "Prob > F" is less than 0.0500, model terms are considered significant. A, B, and A2 are important model terms in this instance. Model terms are not significant if the value is higher than 0.1000. The "Lack of Fit F-value" of 6.24 indicates that there is a 5.46 per cent possibility that noise might be the cause of a "Lack of Fit F-value" this significant. As one may typically anticipate, the "Pred R-squared" of 0.9514 is close to the "Adj R-squared" of 0.9864. The signal-to-noise ratio is measured using "Adeq Precision." A ratio of at least 4 is preferred. An acceptable signal is indicated by the model ratio of 40.7549. The design space can be explored using this model.

TABLE 4: ANOVA FOR QUADRATIC MODEL - RESPONSE 1: PEAK ONE THEORETICAL PLATES

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3.033E+06	5	6.066E+05	175.22	< 0.0001	significant
A-Organic phase	2.471E+06	1	2.471E+06	713.84	< 0.0001	
B-Flow rate	1.580E+05	1	1.580E++05	45.64	0.0003	
AB	10000.00	1	10000.00	2.89	0.1330	
A^2	3.931E+05	1	3.931E+05	113.55	< 0.0001	
B^2	11672.53	1	11672.53	3.37	0.1089	
Residual	24233.72	7	3461.96			
Lack of Fit	19966.92	3	6655.64	6.24	0.0546	not significant
Pure Error	4266.80	4	1066.70			
Cor Total	3.057E+06	12				
Summary of the quadratic model						
Std. Dev.	58.84		R^2	0.9921	Predicted R^2	0.9514
Mean	2414.69		Adjusted R^2	0.9864	Adeq Precision	40.7549

3D and Contour Graph for the Number of Theoretical Plates: From the ANOVA report on the number of theoretical plates, it is clearly stated that the interaction effect of flow rate and mobile phase has a p-value higher than 0.05 so the interaction effect of these terms does not have any significant effect on the number of theoretical plates. By keepinga constant temperature at 25°C, the interaction effect of pH of the organic phase and flow rate were studied from contour and 3D graphs. From the graph, a lower flow rate and lower level of the mobile phase can give a lesser number of theoretical plates **Fig. 2 & 3**.

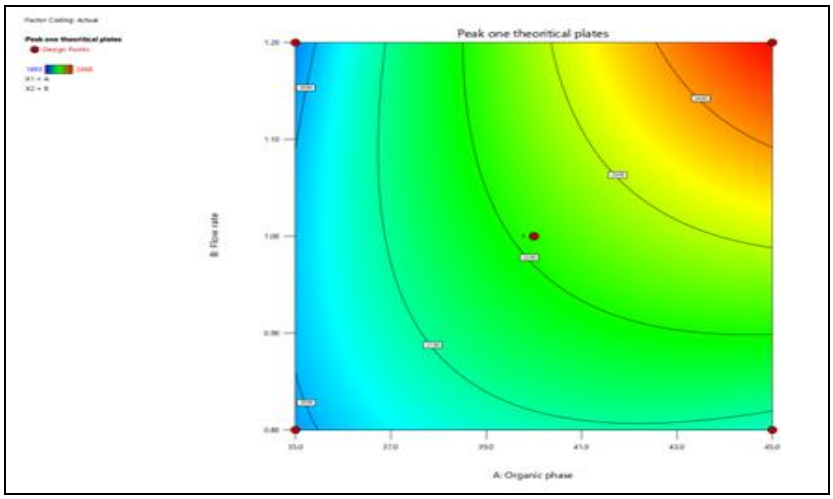

FIG. 2: CONTOUR PLOTS FOR THEORETICAL PLATES AS A FUNCTION OF MOBILE PHASE AND FLOW RATE (CONSTANT TEMPERATURE 30^{O}C)

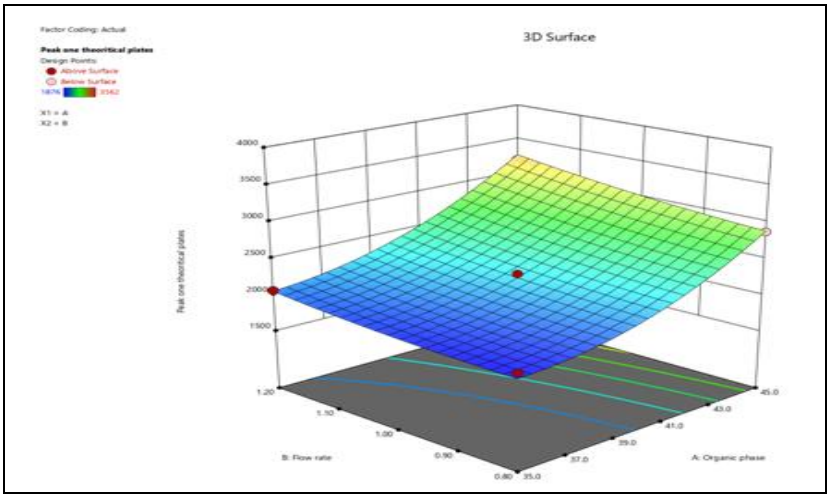

FIG. 3: RESPONSE SURFACE FOR THEORETICAL PLATES AS A FUNCTION OF MOBILE PHASE AND FLOW RATE (CONSTANT TEMPERATURE 30^{O}C)

Statistical analysis of Method Response 2 – Resolution: ANOVA for method response 2: The Design Expert programme provided the analysis of variance (ANOVA) regression parameters for the projected response surface quadratic model for the number of theoretical plates **Table 5**.

The model is important given its Model F-value of 7.86. A "Model F-value" this large could only occur owing to noise with a 0.0001% chance. When "Prob > F" is less than 0.0500, model terms are considered significant. Model terms are not significant if the value is higher than 0.1000. The "Lack of Fit F-value" of 4.77 indicates that there is a 5.26 per cent possibility that noise might be the cause of a "Lack of Fit F-value" this significant. As one may typically anticipate, the "Pred R-squared" of 0.1082 is close to the "Adj R-squared" of 0.7409. The signal-to-noise ratio is measured using "Adeq Precision." A ratio of at least 4 is preferred. An acceptable signal is indicated by the model ratio of 7.9619.

TABLE 5: ANOVA FOR QUADRATIC MODEL - RESPONSE 2: RESOLUTION

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.64	5	0.3285	7.86	0.0086	significant
A-Organic phase	1.22	1	1.22	29.26	0.0010	
B-Flow rate	0.0114	1	0.0114	0.2734	0.6172	
AB	0.0020	1	0.0020	0.0485	0.8321	
A^2	0.1194	1	0.1194	2.86	0.1348	
B^2	0.3321	1	0.3321	7.95	0.0258	
Residual	0.2925	7	0.0418			
Lack of Fit	0.2286	3	0.0762	4.77	0.0826	not significant
Pure Error	0.0639	4	0.0160			
Cor Total	1.94	12				
Summary of the quadratic model						
Std. Dev.	0.2044		R^2	0.8488	Predicted R^2	0.1082
Mean	5.63		Adjusted R^2	0.7409	Adeq Precision	7.9619

3D and Contour Graph for Resolution: From the ANOVA report on resolution, it is clearly stated that the interaction effect of organic phase and flow rate has a p-value higher than 0.05 so the interaction effect of these terms does not have any significant effect on resolution. By keeping a constant temperature at 25°C, the interaction effect of flow rate and organic phase were studied from contour and 3D graphs **Fig. 4** & **5.**

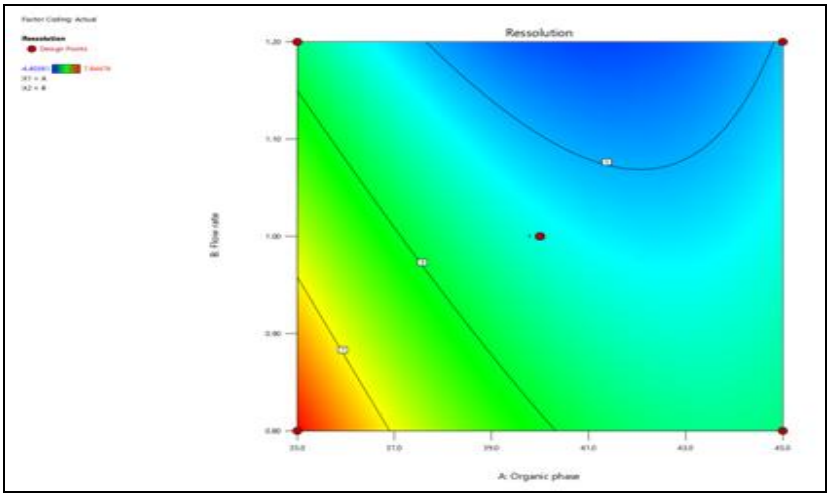

FIG. 4: CONTOUR PLOTS FOR RESOLUTION AS A FUNCTION OF MOBILE PHASE AND FLOW RATE (CONSTANT TEMPERATURE 30^{O}C)

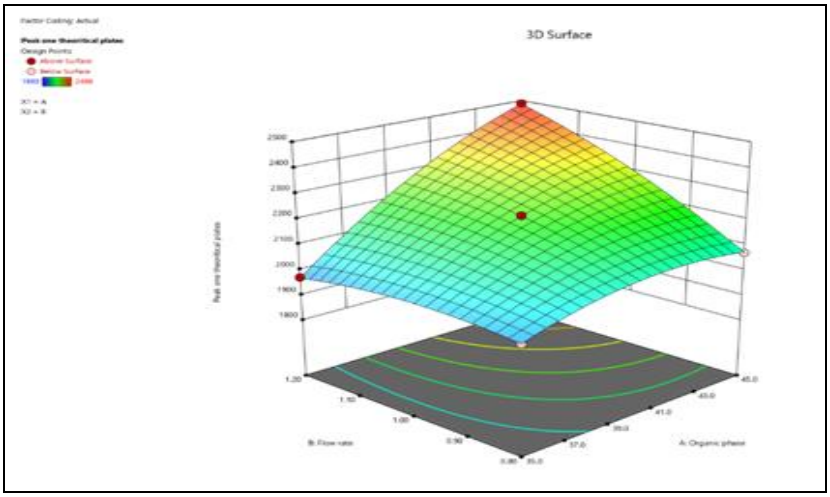

FIG. 5: RESPONSE SURFACE FOR RESOLUTION AS A FUNCTION OF MOBILE PHASE AND FLOW RATE (CONSTANT TEMPERATURE 30^{O}C)

System Suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines. According to ICH guidelines, the plate count should be more than 2000, the tailing factor should be less than 2 and the resolution must be more than 2. All the system-suitable parameters were passed and were within the limits **Table 6.**

TABLE 6: SYSTEM SUITABILITY PARAMETERS FOR AXITINIB & AVELUMAB

S. no.	Parameter	Axitinib	Avelumab
1	Retention time	2.147	3.838
2	Plate count	3287	4511
3	Tailing factor	0.85	0.60
4	Resolution	----	5.85
5	%RSD	0.13	0.34

Assay:

TABLE 7: ASSAY OFAXITINIB & AVELUMAB

Brand	Drug	Avg sample area (n=5)	Std. Conc. (µg/ml)	Sample Conc. (µg/ml)	Label amount (mg)	Std purity	Amount found (µg/ml)	% assay
-	Axitinib	684969	50	50	5	99.8	5.02	100.4
	Avelumab	2781382	200	200	20	99.9	19.99	100.0

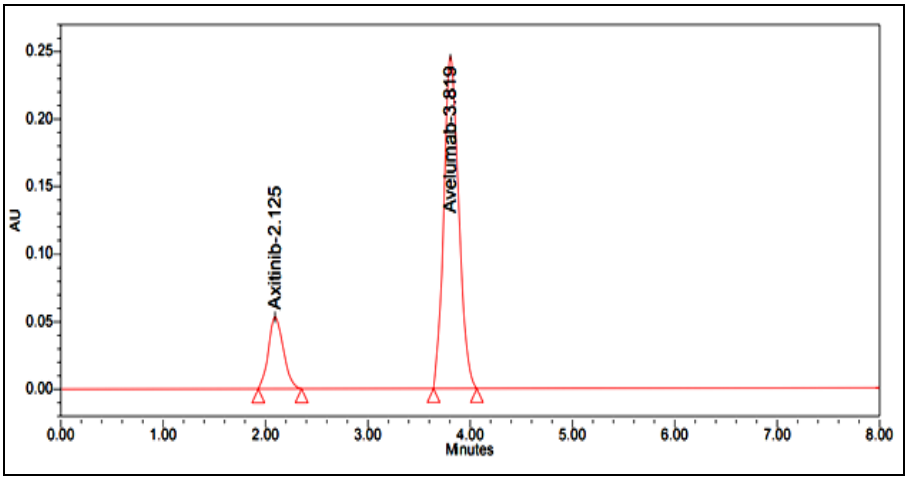

FIG. 6: CHROMATOGRAM OF ASSAY-1

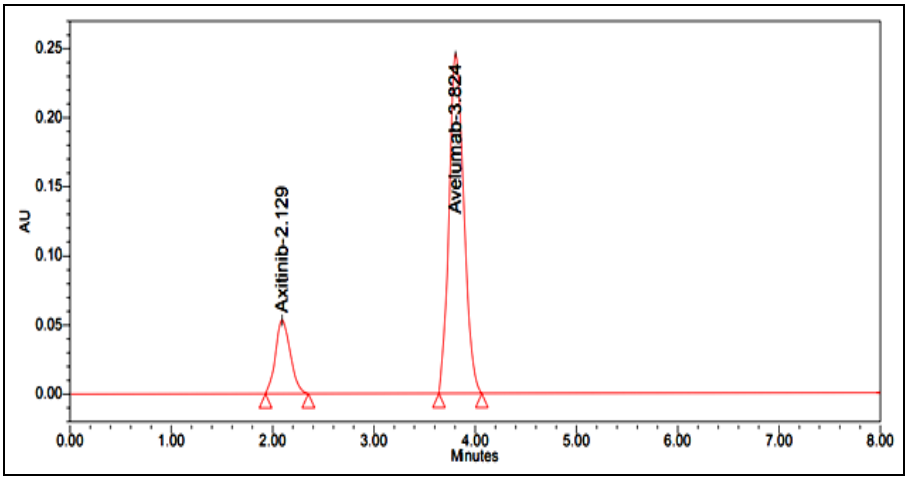

FIG. 7: CHROMATOGRAM OF ASSAY-2

Method Validation: Axitinib and Avelumab had retention durations of 2.141 minutes and 3.832 minutes, respectively. At the retention time of the medications in this approach, we did not detect any interfering peaks in the placebo or blank samples. This approach was therefore said to be specific **Fig.**

8. The devised technique was linear over the concentration ranges of 12.50-75 g/ml for axitinib and 50-300 g/ml for avelumab, with correlation coefficients of 0.9998 and 0.9997, respectively **Table 8 & Fig. 9**. The per cent recovery of the drug was determined to be within 99-100.4 per cent for the accuracy studies at 50, 100, and 150 per cent levels **Table 10**. System, method, and intermediate precision were used, and the results showed that the per cent RSD values were less than 1% **Table 11-13.**

Specificity:

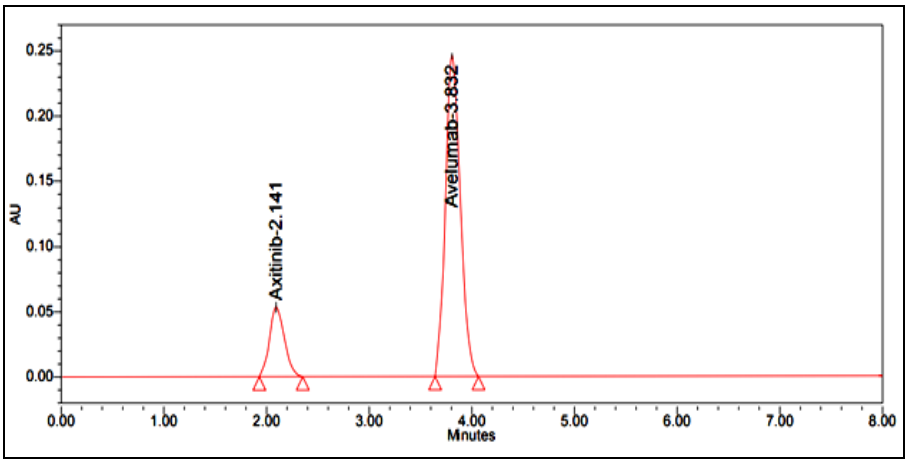

FIG. 8: OPTIMIZED CHROMATOGRAM FOR SPECIFICITY

TABLE 8: RESULTS OF LINEARITY FOR AXITINIB & AVELUMAB

S. no.	Axitinib		Avelumab	
	Conc.(µg/ml)	Peak area	Conc.(µg/ml)	Peak area
1	12.50	173804	50.00	695861
2	25.00	352987	100.00	1397465
3	37.50	513609	150.00	2143507
4	50.00	683457	200.00	2788369
5	62.50	855471	250.00	3401657
6	75.00	1006753	300.00	4122546
Regression equation	y = 13468.75x +7219.11		y =13692.95x + 24543.50	
Slope	13468.75		13692.95	
Intercept	7219.11		24543.50	
R^2	0.99981		0.99970	

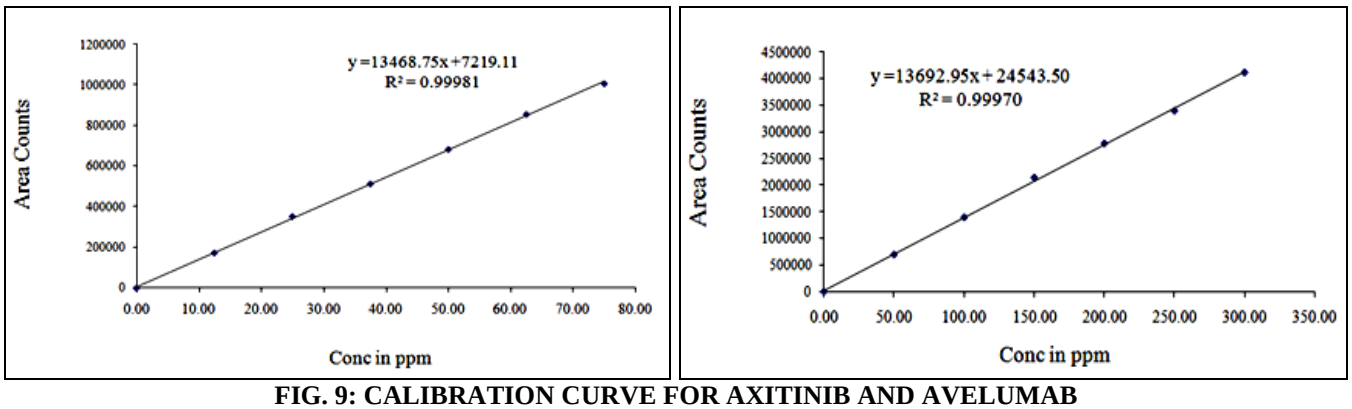

FIG. 9: CALIBRATION CURVE FOR AXITINIB AND AVELUMAB

Accuracy:

TABLE 9: ACCURACY RESULTS OF AXITINIB AND AVELUMAB BY RP-HPLC METHOD

Axitinib					
% Concentration(at specification level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery

50%	342278	2.5	2.51	100.4	100.1
100%	683018	5.0	5.00	100.0	
150%	1023216	7.5	7.49	99.9	
Avelumab					
50%	1396805	10	10.04	100.4	100.0
100%	2786541	20	20.02	100.1	
150%	4155321	30	29.86	99.5	

Precision:

System Precision:

TABLE 10: SYSTEM PRECISION TABLE OF AXITINIB & AVELUMAB

S. no.	Concentration Axitinib (µg/ml)	Area of Axitinib	Concentration of Avelumab (µg/ml)	Area of Avelumab
1.	50	683224	200	2791365
2.	50	682412	200	2767840
3.	50	684079	200	2780396
4.	50	683356	200	2786612
5.	50	682145	200	2793554
6.	50	681718	200	2779685
Mean		682822	2783242	
S.D		880.01	9398.41	
%RSD		0.13	0.34	

Method Precision:

TABLE 11: METHOD PRECISION FOR AXITINIB & AVELUMAB BY RP-HPLC METHOD

S. no.	Area for Axitinib	Area for Avelumab
1	684857	2758946
2	682169	2740351
3	686842	2768912
4	684081	2797455
5	685920	2770630
6	681715	2792413
Average	684264	2771451
Standard Deviation	2032.731	21195.844
%RSD	0.30	0.76

Intermediate Precision:

TABLE 12: INTERMEDIATE PRECISION (DAY VARIATION) FOR AXITINIB AND AVELUMAB BY RP-HPLC METHOD

S. no.	Area for Axitinib		Area for Avelumab	
	Day-1	Day-2	Day-1	Day-2
1	684458	684356	2784654	2766548
2	686119	685017	2773165	2787908
3	681809	680926	2770649	2764153
4	682431	686633	2754126	2780426
5	687152	682974	2742610	2794581
6	684032	682459	2791603	2750322
Average	684334	683728	2769468	2773990
Standard Deviation	2060.629	2026.080	18397.633	16561.680
%RSD	0.30	0.30	0.66	0.60

Degradation Studies: Forced degradation studies of Axitinib and Avelumab were observed in various conditions such as acidic, basic, peroxide, reduction, thermal, photolytic, and hydrolytic conditions. The Axitinib and Avelumab were stable under, reduction, thermal, photolytic and hydrolytic conditions. The drug showed significant degradation in acidic, basic and peroxide conditions represented in **Fig. 11-13**. The results of forced degradation studies are presented in **Table 14.**

TABLE 13: FORCED DEGRADATION RESULTS FOR AXITINIB AND AVELUMAB

% Degradation results	Axitinib					Avelumab				
	Area	% Assay	% Deg	Purity Angle	Purity Threshold	Area	% Assay	% Deg	Purity Angle	Purity Threshold
Control	682896	100.0	0	1.571	10.835	2784957	100.0	0	4.158	15.261
Acid	606224	88.8	11.2	1.563	10.824	2463425	88.5	11.5	4.122	15.247
Alkali	605430	88.6	11.4	1.536	10.871	2447780	87.9	12.1	4.126	15.231
Peroxide	594442	87.0	13.0	1.524	10.878	2379300	85.5	14.5	4.175	15.266
Reduction	670511	98.2	1.8	1.528	10.816	2706321	97.2	2.8	4.111	15.260
Thermal	668563	97.9	2.1	1.552	10.876	2710428	97.4	2.6	4.154	15.219
Photolytic	676914	99.1	0.9	1.542	10.858	2689754	96.6	3.4	4.125	15.337
Hydrolysis	680352	99.6	0.4	1.533	10.869	2714063	97.5	2.5	4.196	15.242

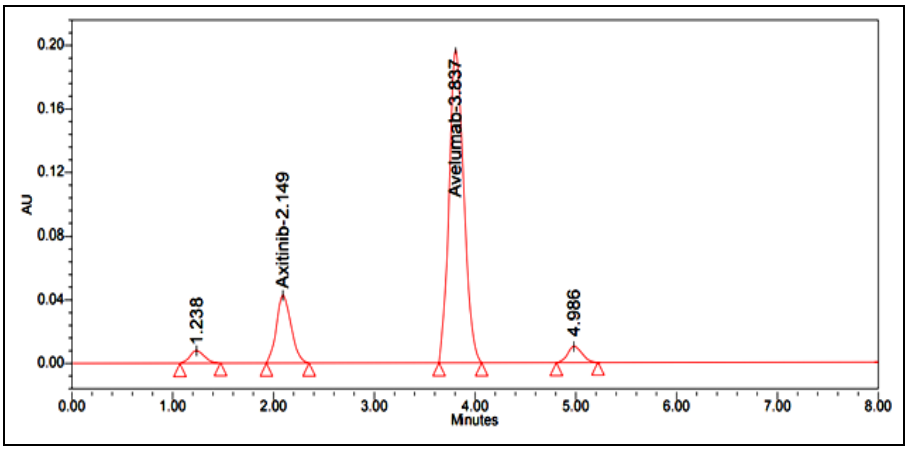

FIG. 10: CHROMATOGRAM OF ACID DEGRADATION

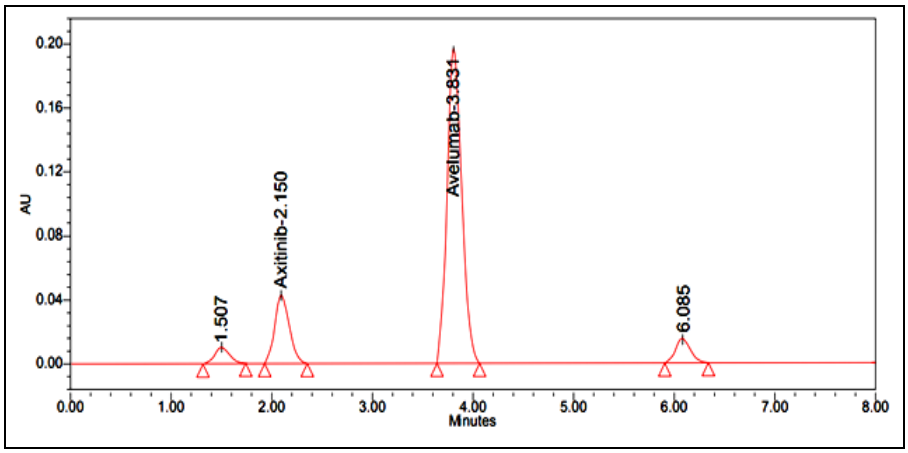

FIG. 11: CHROMATOGRAM OF ALKALI DEGRADATION

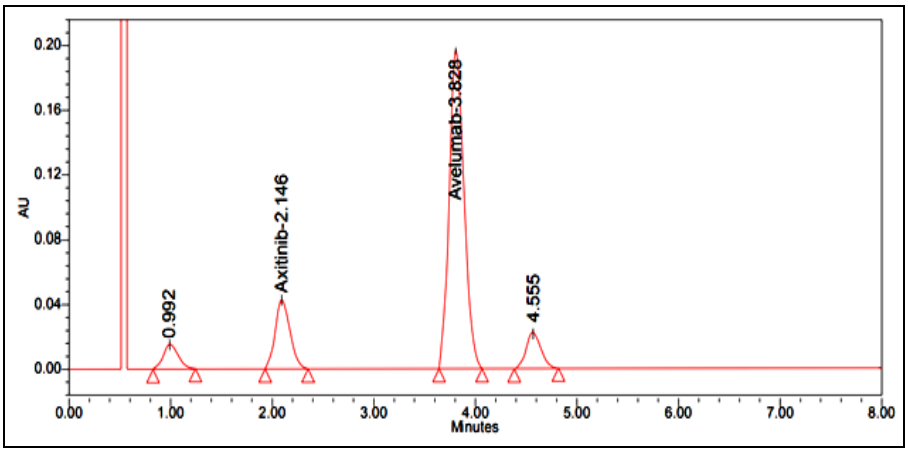

FIG. 12: CHROMATOGRAM OF PEROXIDE DEGRADATION

CONCLUSION: Using an analytical quality-by-design methodology, a straightforward, reliable, and robust RP-HPLC method was created for the quantification of Axitinib and Avelumab. Flow rate and the percentage of organic material in the mobile phase were chosen as the key method parameters (CMPs). Retention time and theoretical plates are the essential quality characteristics. A rotating central composite design was used to methodically tune the CMPs (CCD). Mobile phase acetonitrile: 0.1% TEA (pH adjusted to 2.5 using orthophosphoric acid) in a ratio of 45:55 v/v pumped at a flow rate of 1.2 ml/min constitute the optimal chromatographic conditions. For the drugs Axitinib and Avelumab, the retention times were determined to be 2.141 and 3.832 minutes, respectively. Asymmetry and theoretical plates were discovered to be within the bounds. The created method was accepted by ICH Q2 (R1) recommendations. Studies on drug degradation under different stress conditions revealed that the medication deteriorated more quickly in acidic, basic, and peroxide environments.

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REFERENCES:

1. Choueiri TK, Larkin J, Oya M, Thistlethwaite F, Martignoni M and Nathan P: Preliminary results for avelumab plus axitinib as first-line therapy in patients with advanced clear-cell renal-cell carcinoma (JAVELIN Renal 100): an open-label, dose-finding and dose-expansion, phase 1b trial. Lancet Oncol [Internet] 2018 Apr 1 [cited 2023; 19(4): 451–60. Available from: https://pubmed.ncbi.nlm.nih.gov/29530667/
2. Motzer RJ, Penkov K, Haanen J, Rini B, Albiges L and Campbell MT: Avelumab plus Axitinib versus Sunitinib for Advanced Renal-Cell Carcinoma. New England Journal of Medicine [Internet]. 2019 Mar 21 [cited 2023 Aug 17]; 380(12): 1103–15. Available from: https://www.nejm.org/doi/full/10.1056/NEJMoa1816047
3. Harish V, Almalki WH, Alshehri A, Alzahrani A, Gupta MM and Alzarea SI: Quality by Design (QbD) Based Method for Estimation of Xanthohumol in Bulk and Solid Lipid Nanoparticles and Validation. Molecules [Internet]. 2023 Jan 1 [cited 2023 Aug 17]; 28(2). Available from: /pmc/articles/PMC9864017/
4. Bhattacharya S and Bhattacharya S: Central Composite Design for Response Surface Methodology and Its Application in Pharmacy. Response Surface Methodology in Engineering Science [Internet]. 2021 Jan 28 [cited 2023 Aug 17]; Available from: https://www.intechopen.com/chapters/74955
5. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human use ICH Harmonised Tripartite Guideline Validation of Analytical Procedures: Text and Methodology Q8 (R2).
6. Kumar P, Mangla B, Beg S, Afzal O, Saleh Alfawaz Altamimi A and Almalki WH: Optimization and validation of stability indicating RP-HPLC method for the quantification of gefitinib in bulk drug and nanoformulations: An application towards *in-vitro* and *ex-vivo* performance evaluation. Arabian Journal of Chemistry 2022; 15(12): 104333.
7. Monks K, Molnár I, Rieger HJ, Bogáti B and Szabó E: Quality by Design: Multidimensional exploration of the design space in high performance liquid chromatography method development for better robustness before validation. J Chromatogr A [Internet]. 2012 Apr 6 [cited 2023 Aug 17]; 1232: 218–30. Available from: https://pubmed.ncbi.nlm.nih.gov/22226460/
8. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human use ICH Harmonised Tripartite Guideline Validation of Analytical Procedures: Text and Methodology Q9 (R1).
9. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human use ICH Harmonised Tripartite Guideline Validation of Analytical Procedures: Text and Methodology Q2 (R1).

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