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Research Article

A STUDY OF METHOD DEVELOPMENT, VALIDATION, AND FORCED DEGRADATION FOR SIMULTANEOUS QUANTIFICATION OF NIVOLUMAB AND RELATLIMAB IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT

To gain some additional advantages over other methods already created for this combination, a rapid and stability-indicating reversed phase high-performance liquid chromatography (RP-HPLC) method was developed for simultaneous quantification of Nivolumab and Relatlimab in their combined dosage form. According to USP guidelines for accuracy, precision, specificity, linearity, solution stability, robustness, sensitivity, and system appropriateness, the method was validated. According to the International Conference on Harmonisation (ICH), the forced degradation study was verified. According to the International Conference on Harmonisation (ICH), the forced degradation study was verified. For this, an isocratic condition of mobile phase was maintained on RP C18 (octadecylsilane (ODS), 150 4.6 mm, 5 m, Phenomenex Inc.) column at room temperature. The mobile phase consisted of phosphate buffer (pH 6.8) and acetonitrile at a ratio of 65:35, v/v, With correlation coefficient (R2) values of 0.999 and 1.0 for Nivolumab and Relatlimab, respectively, the method demonstrated excellent linear response and was within the correlation coefficient's range (R2≥ 0.995). Two medication's percent recoveries fell within the acceptable range of (99.60-99.82.%).The novel method's intra- and inter-day precision assessments showed that the relative standard deviation (%RSD) was less than the 2.0 maximum permitted limit. According to ICH guidelines, the drug product was forced to degrade in order to determine its stability-indicating ability and learn more about the degradation pathways, degradation products, and how the quality of a drug substance and drug product varies over time under different stressful conditions. Relatlimab degradation was within the acceptable range (5-20%, per ICH guidelines), however Nivolumab demonstrated a 20% degradation in oxidation and basic condition.

Keywords: RP-HPLC, stability indicating, Nivolumab , Relatlimab.

INTRODUCTION

Relatlimab is a human IgG4 monoclonal antibody and novel immune checkpoint inhibitor that targets lymphocyte activation gene-3 (LAG-3). Nivolumab is indicated to treat unresectable or metastatic melanoma, melanoma as adjuvant treatment, resectable or metastatic non-small cell lung cancer[1]. The combination of relatlimab and nivolumab can cause severe and fatal immune-mediated adverse reactions, including pneumonitis, colitis, hepatitis, myocarditis, and hypophysitis. once- monthly administration alongside nivolumab, steady-state concentrations of relatlimab were reached after 16 weeks. The ligands PD-L1 and PD-L2 bind to the PD-1 receptor on T-cells, inhibiting the action of these cells.6 Tumor cells express PD-L1 and PD-L2.6 Nivolumab binds to PD-1, preventing PD-L1 and PD-L2 from inhibiting the action of T-cells, restoring a patient's tumor-specific T-cell response [2].

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Structure of Relatlimab

To ensure the quality of tablets or capsules in terms of assay, content homogeneity, and dissolving, it is imperative to develop an analytical method for evaluating pharmaceuticals in pharmaceutical dosage form[3]. The United States Pharmacopoeia (USP) and the British Pharmacopoeia (BP) both have standards for evaluating these two medications separately. Here, we make an effort to create a reversed phase (RP)-HPLC method that is quick, efficient, robust, and provides a sufficient amount of data for validation parameters. Drug pKa was looked into first ,Nivolumab and Relatlimab have pKas of 9.5 and 4.85, respectively. As a general guideline, the mobile phase pH is chosen two units above or below the drug's pKa value. If we take Nivolumab pKa into account, we can't choose a pH above 9.5 because it will harm column silica beds. Again, we could set the pH of the mobile phase for two Relatlimab units below its pKa (4.8), but at that acidic pH, ibuprofen is completely undissociated, leading to a strong hydrophobic interaction with the silica bed and, as a result, longer retention of this medication.

MATERIALS AND METHOD

MATERIALS

The Reference standard of Nivolumab and Relatlimab pure drugs was purchased from Harish Lab, Vellore. The commercial product of Nivolumab and Relatlimab Injectable solution was bought from Local market.Distilled water, Acetonitrile, Phosphate buffer, Acetonitrile, Potassium dehydrogenate Ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

HPLC SYSTYEM

The analysis was conducted using a high-performance liquid chromatographic system (Shimadzu-Prominence, Japan), which was furnished with an auto sampler (Model SIL-20AC HT) and UV-visible detector (Model SPD 20A). Data were captured with the aid of LC-solution software. The standard and samples were analysed using an analytical RP C18 column (octadecylsilane (ODS), 150 4.6 mm, 5, Phenomenex Inc., Japan).

Preparation of Standard stock solutions:

Accurately weighed 24mg of Nivolumab, 8mg of Relatlimab transferred to individual 50ml volumetric flasks separately. 3/4th of diluents was added to both flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1 and 2. (480µg/ml of Nivolumab and 160µg/ml of Relatlimab) [4].

Preparation of Standard working solutions (100% solution):

1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (48µg/ml Nivolumab of and 16µg/ml of Relatlimab)[5].

Validation:

System suitability parameters:

The system suitability parameters were determined by preparing standard solutions of Nivolumab (48ppm) and Relatlimab (16ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.The % RSD for the area of six standard injections results should not be more than 2%.

Specificity:

Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific

Precision:

1 vial transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (2400µg/ml of Nivolumab and 800µg/ml of Relatlimab) 0.2ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (48µg/ml of Nivolumab and 16µg/ml of Relatlimab).[6]

Linearity:

Accurately weighed 24 mg of Nivolumab, 8 mg of Relatlimab and transferred to individual 50 ml volumetric flasks separately. 3/4 th of diluents was added to both flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1 and 2. (480µg/ml of Nivolumab and 160µg/ml of Relatlimab)[7].

Accuracy:

Accurately weighed 24 mg of Nivolumab, 8 mg of Relatlimab and transferred to individual 50 ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1 and 2.

LOD Preparation:

0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Nivolumab, Relatlimab,

solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents.

Degradation studies:

Oxidation:

To 1 ml of stock solution of Nivolumab and Relatlimab, 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 48µg/ml& 16µg/ml solution and 10.0µl were injected into the system and the chromatograms were recorded to assess the stability of sample.[8]

Acid Degradation Studies:

To 1ml of stocks solution Nivolumab and Relatlimab, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 48µg/ml& 16µg/ml solution and 10.0µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample[9].

Alkali Degradation Studies:

To 1 ml of stock solution Nivolumab and Relatlimab, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 48µg/ml& 16µg/ml solution and 10.0µl were injected into the system and the chromatograms were recorded to assess the stability of sample.[10]

Dry Heat Degradation Studies:

The standard drug solution was placed in oven at 105°C for 6h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 48µg/ml& 16µg/ml solution and 10.0µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.[11]

Photo stability studies:

The photochemical stability of the drug was also studied by exposing the 2400µg/ml & 800µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200-Watt hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 48µg/ml& 16µg/ml solutions and 10.0µl were injected into the system and the chromatograms were recorded to assess the stability of sample.[12]

Neutral degradation Studies:

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at temperature of 60ºC.For HPLC study, the resultant solution was diluted to 48µg/ml& 16µg/ml solution and 10.0µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.[13]

RESULTS AND DISCUSSION

System suitability:

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

Validation:

Retention times of Nivolumab and Relatlimab were 2.132min and 2.696min respectively. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific

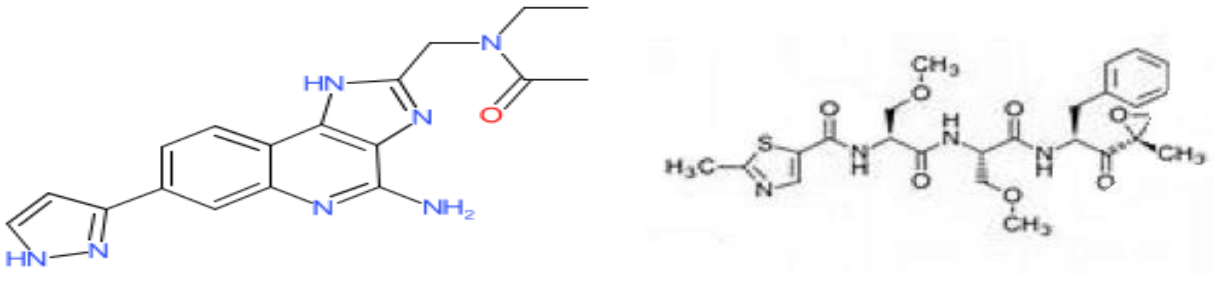
Linearity:

Six linear concentrations of Nivolumab (12-72µg/ml) and Relatlimab (4-24µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Nivolumab was y = 19668x + 5224.3 and of Relatlimab was y = 18997x + 186.57. Correlation coefficient obtained was 0.999 for the two drugs

ACCURACY

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.60% and 99.82% for Nivolumab and Relatlimab respectively.

Figure 1: Structure of Relatlimab and Nivolumab



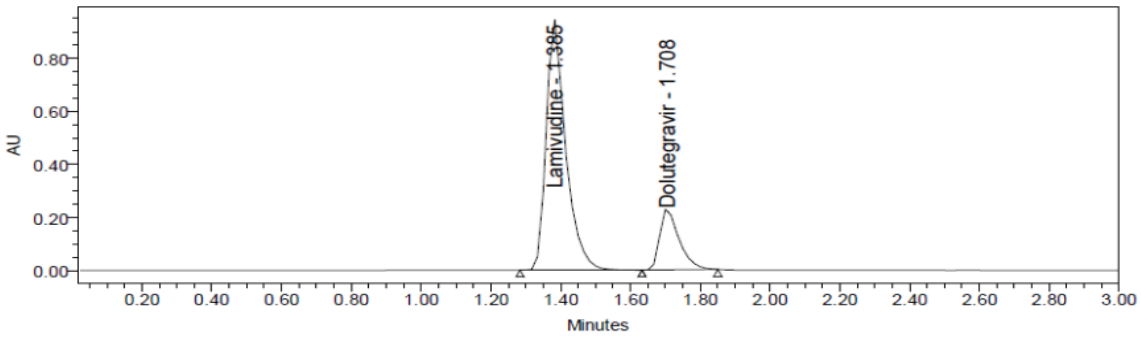


Figure 2: Chromatogram of blank.

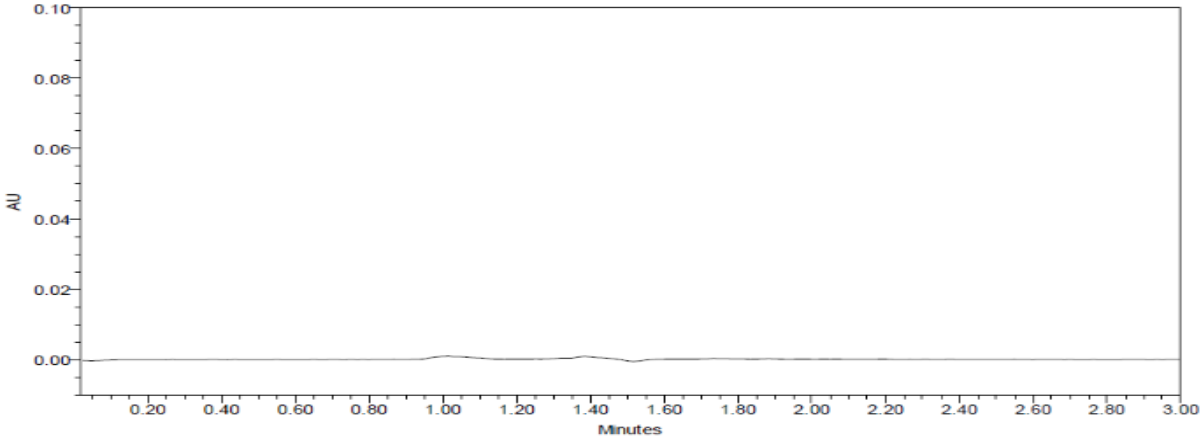


Figure 3: Chromatogram of placebo

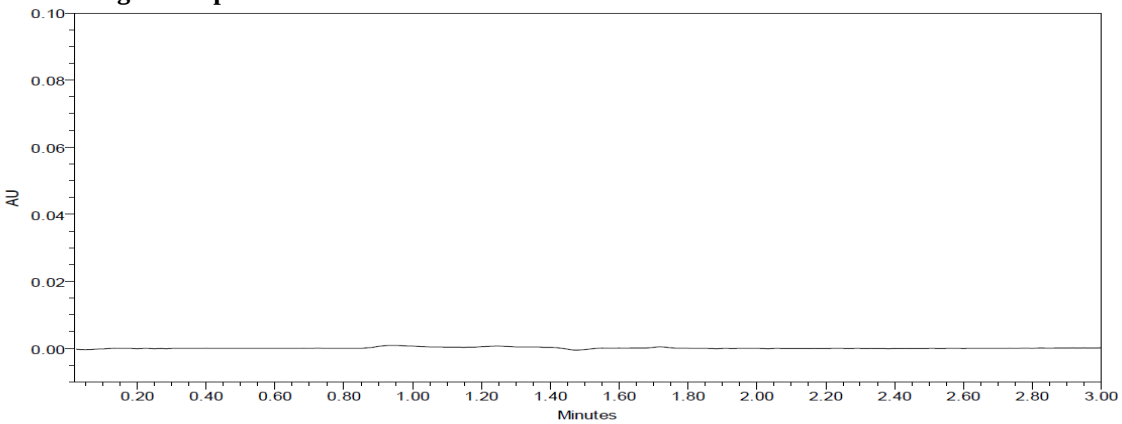


Figure 4: Optimized Method.

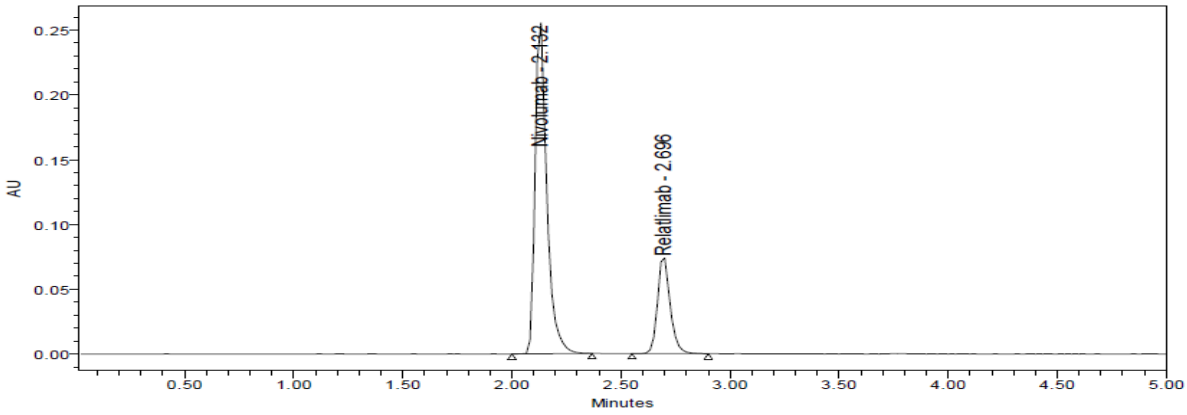


Figure 5: Calibration curve of Nivolumab.

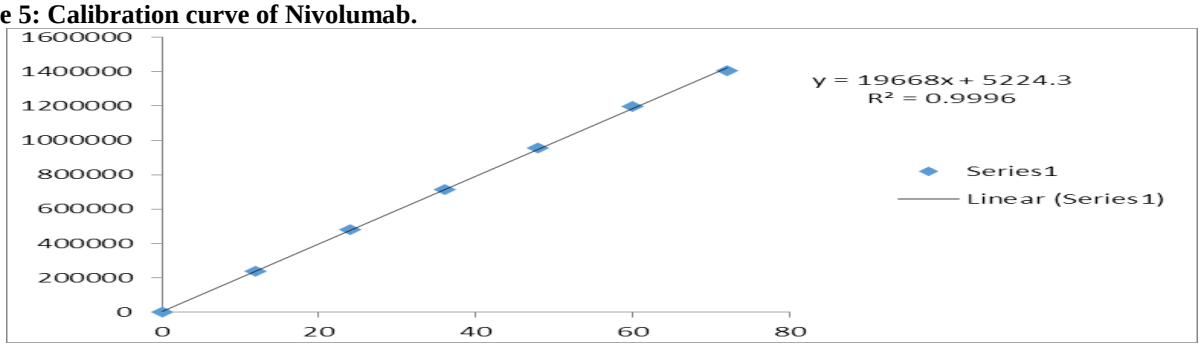
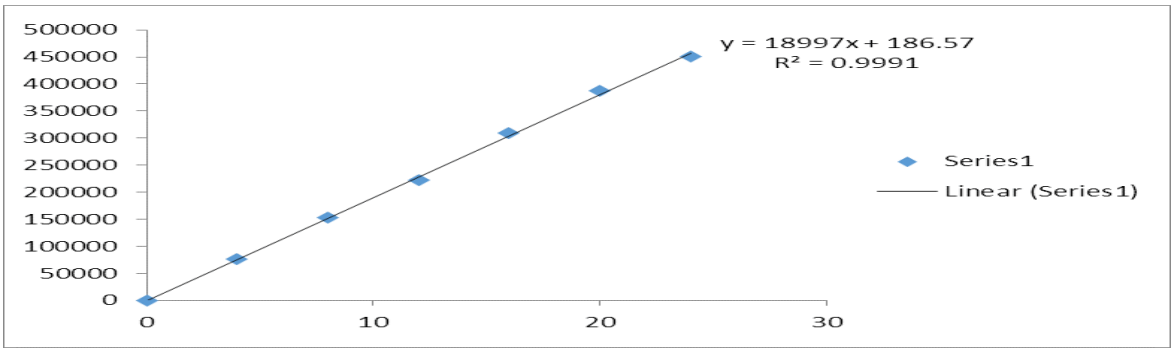


Figure 6: Calibration curve of Relatlimab



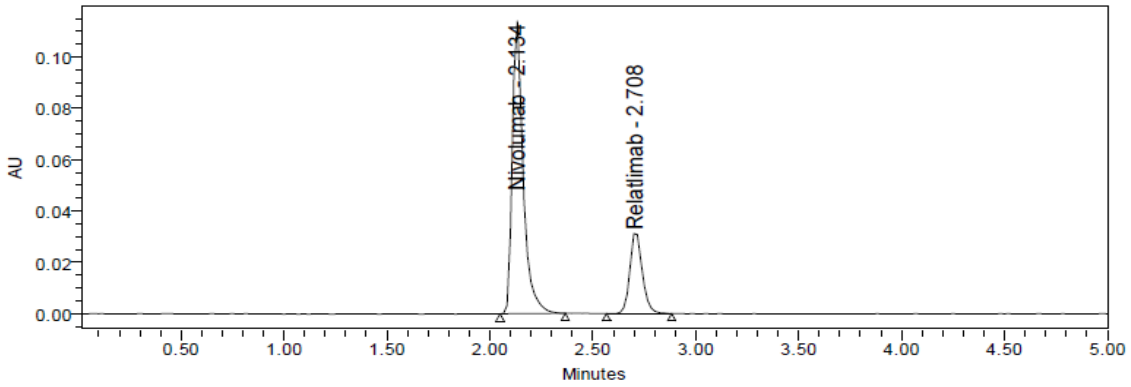


Figure 7: Linearity 50% Chromatogram of Nivolumab and Relatlimab.

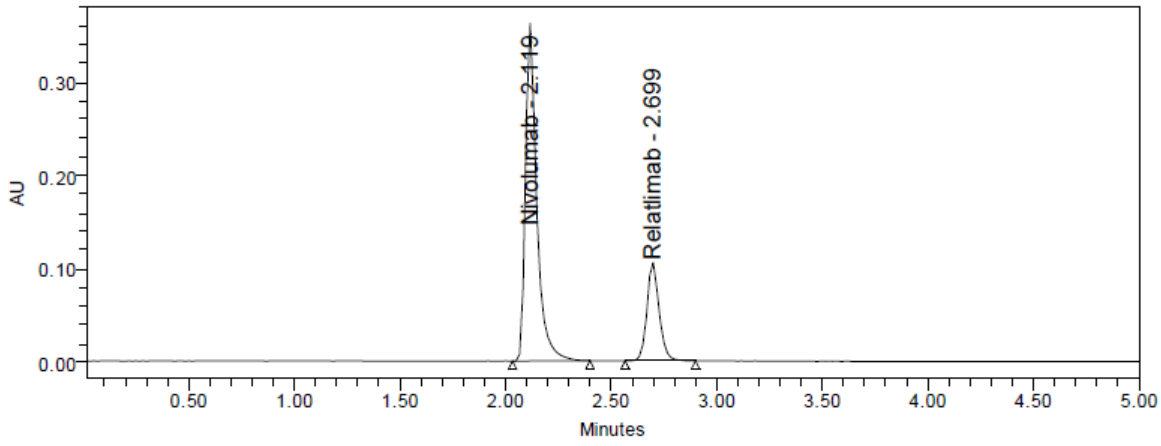


Figure 8: Accuracy % Chromatogram of Nivolumab and Relatlimab

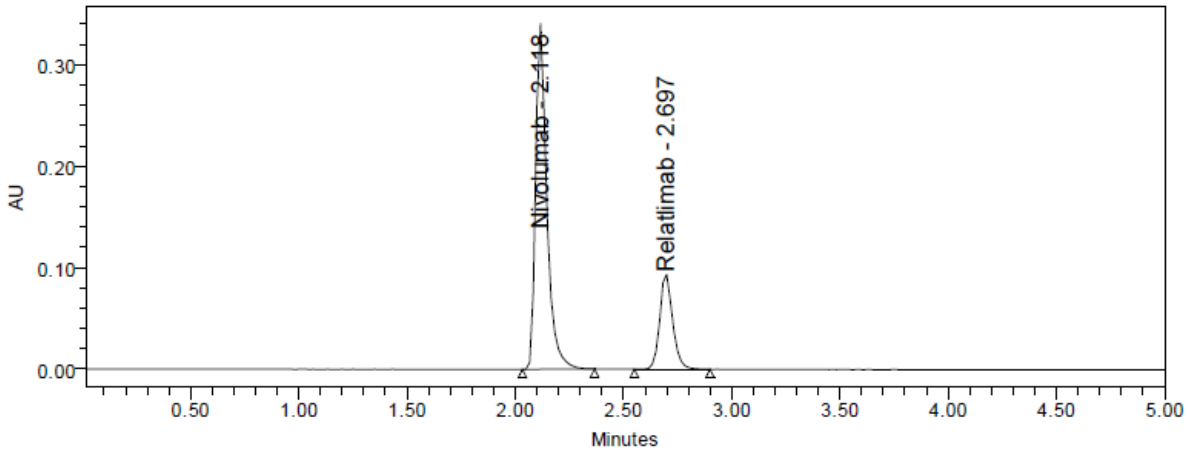


Table 1: Degradation data:

Type of degradation	Nivolumab			Relatlimab		
	AREA	%Recovered	% Degraded	Area	%Recovered	% Degraded
Acid	912743	95.02	4.98	290894	95.32	4.68
Base	895278	93.20	6.80	286181	93.78	6.22
Peroxide	890733	92.73	7.27	287050	94.06	5.94
Thermal	942279	98.10	1.90	289400	94.83	5.17
Uv	931702	96.99	3.01	293926	96.32	3.68
Water	953277	99.24	0.76	303625	99.50	0.50

Figure 9: Thermal, Uv degradation chromatogram

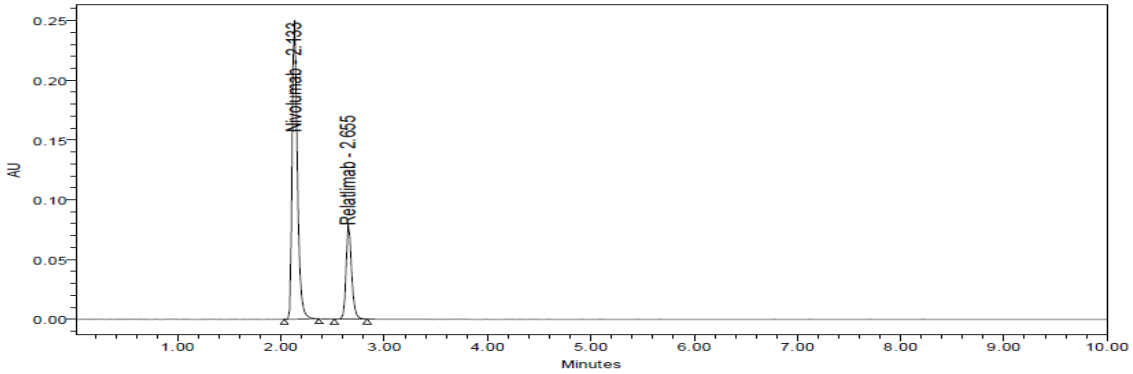
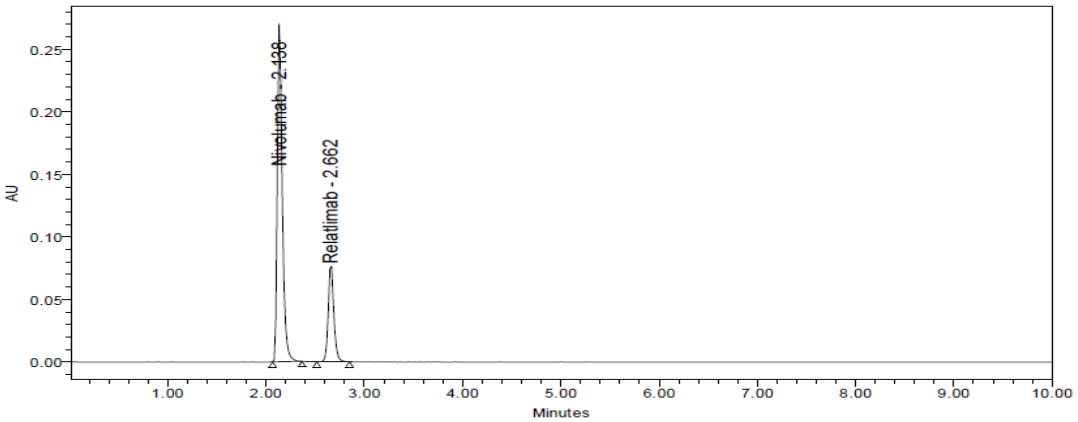


Figure 10: Water degradation chromatogram, Base Degradation



Discussion:

In this way, a novel and reliable approach for the simultaneous measurement of in their Nivolumab and Relatlimab solid dose form was created. We rigorously validated it while adhering to the ICH, USP, and Food and Drug Administration (FDA) standards. The procedure was primarily intended for the measurement of in tablets Nivolumab and Relatlimab or capsules. Additionally, this HPLC approach was used to detect and quantitatively analyse the amount of Nivolumab and Relatlimab that degraded in a variety of environments, including alkaline, acidic, oxidative, and reduced conditions.

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References

1. MacNair J.E., Patel K.D And Jorgenson J.W., Ultra pressure reversed phase capillary Liquid Chromatography: Isocratic and gradient elusion using columns packed with 1µm particles, *Anal. Chem*. 71, 1999, 700–708.
2. Wu N., Lippert J.A. And Lee M.L, *et al.* "Practical aspects of ultrahigh pressure capillary liquid *chromatography*, *J. Chromatogr*, A 911, 2001, 1–12.
3. Swartz M. E. Ultra Performance Liquid Chromatography (HPLC): An Introduction, Separation Science Re-Defined, LCGC Supplement, 2005, 8-11.
4. Jerkovich A.D, Mellors J.S and Jorgenson J.W, *et al.* The use of micrometer-sized particles in ultrahigh pressure liquid chromatography. LCGC. 21(7), 2003, 600–610.
5. MacNair J.E, Lewis K.C And Jorgenson J.W, *et al.* Ultrahigh-pressure reversed-phase liquid chromatography in packed capillary columns. *Anal. Chem*. 69, 1997, 983–989.
6. Van Deemter JJ, Zuiderweg EJ, Klinkenberg A, *et al*. Longitudinal diffusion and resistance to mass transfer as causes of non ideality in chromatography. *Chem. Eng. Sci*. 5, 1956, 271–289.
7. Lars Y. and Honore H.S. On-line turbulent-flow chromatography–high-performance liquid chromatography–mass spectrometry for fast sample preparation and quantitation *J. Chromatogr*., A 1020, 2003, 59–67.
8. McLoughlin D.A, Olah T.V and Gilbert J.D, *et al.* A direct technique for the simultaneous determination of 10 drug candidates in plasma by Liquid Chromatography atmospheric pressure chemical ionization mass spectrometry interfaced to a prospect solid-phase extraction system. *J. Pharm. Biomed. Anal.* 15, 1997, 1893–1901.
9. Goodwin L, White SA, Spooner N, *et al*. Evaluation of ultra-performance liquid chromatography in the bioanalysis of small molecule drug candidates in plasma. *J. Chromatogr. Sci*. 45(6), 2007, 298–304.
10. Swartz M, Ultra Performance Liquid Chromatography. LCGC. 23(1), 2005, 46–53.
11. Broske A.D, *et al.*, Agilent Technologies application note 2004, 5988-9251EN.
12. Michael E. Swartz and Brian J. Murphy, *et al*. Ultra performance liquid chromatography: tomorrow's HPLC technology today as published in LPI- June 2004.
13. Michael E. Swartz, HPLC: An Introduction and Review Waters Corporation, Milford, Massachusetts, USA, *Journal of Liquid Chromatography & Related Technologies*, 28, 2005, 1253–1263.

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