e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:06/Issue:11/November-2024 Impact Factor- 8.187 www.irjmets.com

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF NIVOLUMAB AND IPILIMUMAB

Bogoju Kranthi*1, Dr. M. Ajitha*2

*1,2Department Of Pharmaceutical Analysis, Centre For Pharmaceutical Sciences, UCEST, JNTUH, Hyderabad, India.

ABSTRACT

An HPLC technique was devised to measure Nivolumab and Ipilimumab concurrently in injectable form. Chromatography was performed using an Inertsil column with dimensions of 250 mm × 4.6 mm × 5 µm. In the mobile phase, acetonitrile and buffer were combined in a ratio of 60:40. A temperature of 30°C was maintained for the column and a flow rate of 1 mL/min was maintained. The best frequency for the detection was determined to be 246nm. The retention times of nivolumab were 2.121 minutes and those of ipilimumab were 2.886 minutes. These are the regression equations for Nivolumab (y = 26340x + 2689.7) and Ipilimumab (y = 20383x + 3595.7). The accuracy was good, with Nivolumab having a low percentage RSD value of 0.7% and Ipilimumab having a low percentage RSD value of 0.9%. The accuracy of the approach was validated by the respective recoveries of 99.47% and 99.66% for ipilimumab and nivolumab. Ipilimumab had similar values of 0.12 µg/mL and 0.37 µg/mL, whereas Nivolumab had LOQ and LOD of 0.22 µg/mL and 0.07 µg/mL, respectively.

Keywords: Nivolumab, Ipilimumab, RP-HPLC And Method Validation.

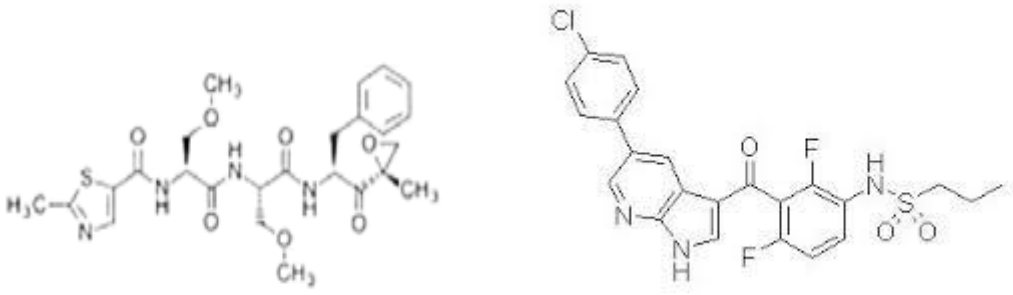
I. INTRODUCTION

Nivolumab (Opdivo) and ipilimumab (Yervoy) are immunotherapy medicines used together to treat metastatic melanoma. The combination of these two medications has the potential to be more effective than either alone in reducing tumours and extending patients' lives.

Nivolumab is a targeted treatment medication known as an immune checkpoint inhibitor. It is a monoclonal antibody that binds to the PD-1 protein found on the surface of T lymphocytes. It works by preventing cancer cells from inhibiting the immune system. This permits the immune system to attack and eliminate cancer cells.

Nivolumab is indicated for treating unresectable or metastatic melanoma, melanoma as adjuvant treatment, metastatic non-small cell lung cancer, small cell lung cancer, advanced renal cell carcinoma, classical Hodgkin lymphoma, squamous cell carcinoma of the head and neck, urothelial carcinoma, microsatellite instability-high or mismatch repair deficient metastatic colorectal cancer, hepatocellular carcinoma, and esophageal cancer.

Ipilimumab is a monoclonal antibody that attaches itself to the T cell protein CTLA-4. It functions by preventing the immune system from being suppressed by cancer cells. This enables the cancer cells to be attacked and destroyed by the immune system.



Nivolumab **Ipilimumab**

Figure-1: Structure of Nivolumab and ipilimumab

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:06/Issue:11/November-2024 Impact Factor- 8.187 www.irjmets.com

II. MATERIALS AND REAGENTS

Ipilimumab and Nivolumab pure drugs (API)were aquired from Spectrum Pharma resreach solutions. Combination Nivolumab and Ipilimumab injections (Opdivo/Yervoy) received from local market hyderabad, Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem

Instrumentation:

Analysis was conducted by using WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software.

III. METHODOLOGY

Chromatographic conditions:

Column Used : Inertsil -250x4.6mm, 5µ
Buffer used : (0.1%) 0.1% Orthophosphoric acid
Mobile phase : 0.1% Orthophosphoric acid: Acetonitrile (60:40A)
Flow rate : 1 ml/min
Diluent : Water: ACN (50:50)
Wavelength : 246nm
Temperature : 30° C
Injection Volume : 10µl

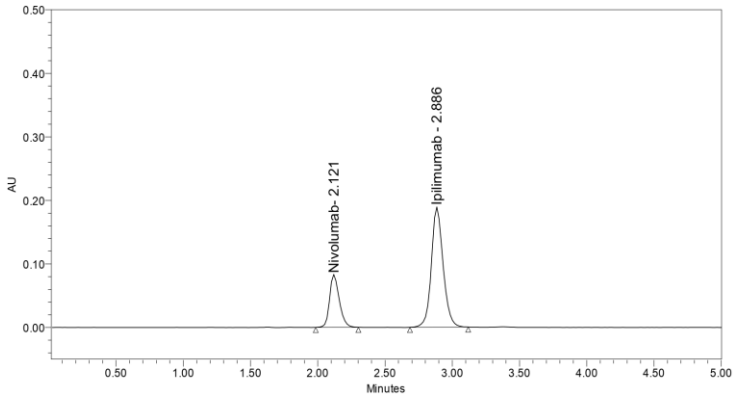


Figure-2: Optimized chromatogram

Preparation of buffer:

0.1%OPA Buffer: 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50

Preparation of Standard stock solutions: Accurately weighed 10 mg of Ipilimumab, 5mg of Nivolumab and transferred to 50ml volumetric flask and 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (200µg/ml of Ipilimumab and 100µg/ml of Nivolumab)

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. (20µg/ml of Ipilimumab and 10µg/ml of Nivolumab)

Preparation of Sample stock solutions: 10ml ampule contains (10mg/ml of Ipilimumab and 5mg/ml of Nivolumab transferred 1ml into a 50 ml volumetric flask, 10ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (200µg/ml of Ipilimumab and 100µg/ml of Nivolumab)

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:06/Issue:11/November-2024 Impact Factor- 8.187 www.irjmets.com

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (20µg/ml of Ipilimumab and 10µg/ml of Nivolumab)

IV. METHOD VALIDATION

System suitability parameters:

The system suitability parameters were determined by preparing standard solutions of Ipilimumab (20ppm) and Nivolumab (10ppm) 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%.

Table 1: System suitability data

Injection	Nivolumab			Ipilimumab			
S no	Retention time	Plate Count	Tf	Retention time	Plate Count	Tf	Rs
1	2.119	4213	1.23	2.882	5302	1.10	5.2
2	2.120	4202	1.24	2.885	5295	1.10	5.2
3	2.121	4151	1.23	2.885	5336	1.11	5.1
4	2.121	4013	1.20	2.885	5265	1.10	5.2
5	2.121	4159	1.23	2.885	5300	1.11	5.2
6	2.122	4226	1.18	2.886	5262	1.11	5.2

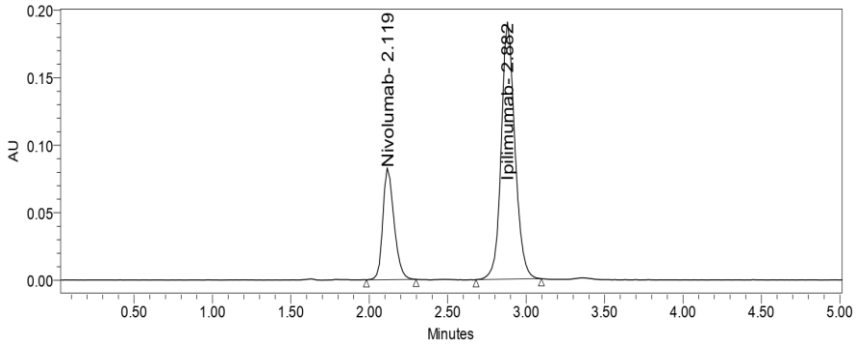


Figure 3: System suitability chromatogram

Specificity: Checking of the interference in the optimized method. We have not found interfering peaks in blank and placebo at retention times of Nivolumab and ipilimumab in this method. So, this method was said to be specific. Figure 4 Displays a representative chromatogram.

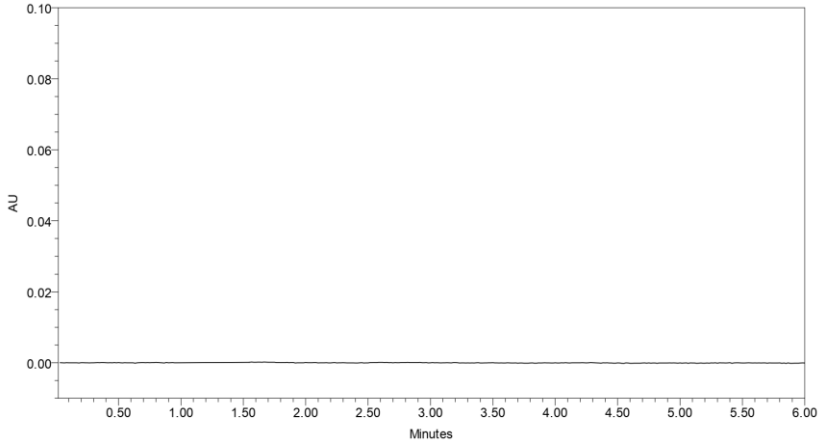


Figure 4: Specificity chromatogram

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:06/Issue:11/November-2024 Impact Factor- 8.187 www.irjmets.com

Linearity:

Table 2: Linearity of Nivolumab and ipilimumab

S.no	Concentration Nivolumab (µg/ml)	Response	Concentration Ipilimumab (µg/ml)	Response
1	0	0	0	0
2	2.5	66504	5	105127
3	5	137046	10	206363
4	7.5	196702	15	308163
5	10	269026	20	414910
6	12.5	336256	25	515553
7	15	393466	30	611666

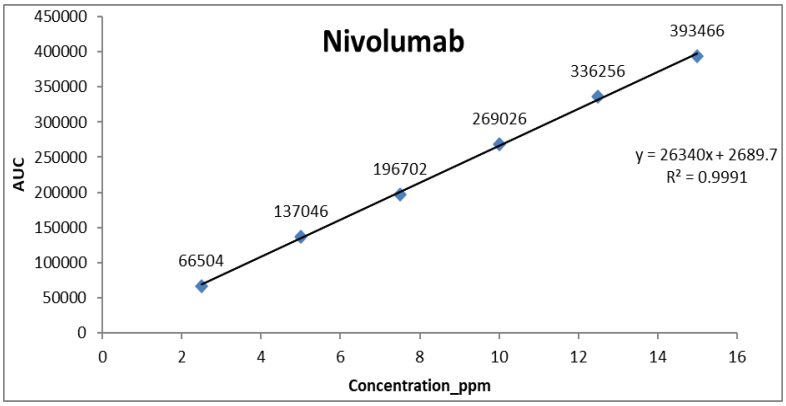


Figure 5: Calibration curve of Nivolumab

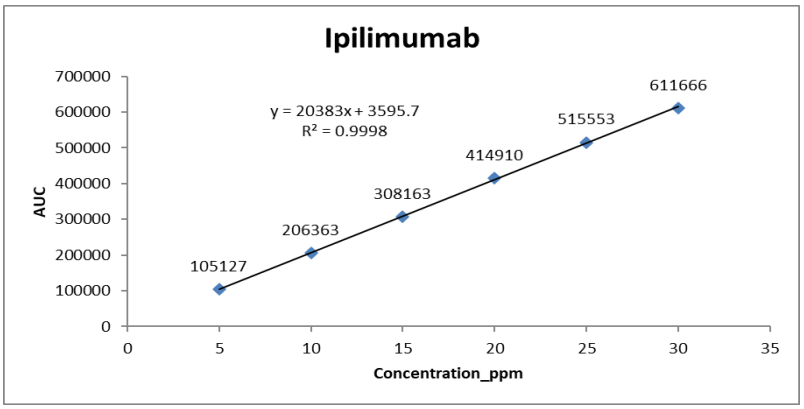


Figure 6: Calibration curve of Ipilimumab

Accuracy (Percentage recovery): Three concentrations 50%, 100%, 150%, were injected in a triplicate manner and amount Recovered and % Recovery was displayed in Table 3.

Table 3: Recovery data of Nivolumab and Ipilimumab

	Nivolumab			Ipilimumab		
% stage	Amount taken	Recovered	% of recovery	Amount taken	recovered	% of recovery
50%	10	9.84	98.39	5	4.95	98.98
	10	10.02	100.17	5	4.89	97.89
	10	9.93	99.33	5	4.96	99.12
100%	20	19.88	99.40	10	9.89	98.90
	20	19.89	99.47	10	9.96	99.65

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:06/Issue:11/November-2024 Impact Factor- 8.187 www.irjmets.com

	20	20.00	100.00	10	10.05	100.51
150%	30	30.15	100.51	15	14.92	99.44
	30	29.72	99.06	15	14.85	98.99
	30	29.67	98.89	15	14.97	99.82
Mean % recovery	99.47			99.25		

Precision:

System precision: The% RSD was found to be 0.7 and 0.9%

Table 4: System precision

Sr. No.	Nivolumab	Ipilimumab
1	266745	420736
2	262464	414027
3	266546	412507
4	266574	416221
5	265755	413241
6	263554	420652
Mean	265273	416231
Std. Dev.	1819.8	3674.4
%RSD	0.7	0.9

Intraday precision (Repeatability): Intraday Precision was performed and % RSD for Nivolumab and Ipilimumab were found to be 0.9% and 0.6% respectively.

Table 5: Repeatability results for Nivolumab and Ipilimumab

Sr. No.	Nivolumab	Ipilimumab
1	261654	414029
2	263655	416130
3	268556	418628
4	263656	414117
5	266475	420048
6	265465	413945
Mean	264910	416150
Std. Dev.	2441.3	2639.3
%RSD	0.9	0.6

Inter day precision: Inter day precision was performed with 24 hrs time lag and the %RSD Obtained for Nivolumab and Ipilimumab were 0.9% and 0.4%.

Table 6: Inter day precision results for Nivolumab and Ipilimumab.

Sr. No.	Nivolumab	Ipilimumab
1	263657	412169
2	260465	413932
3	265455	415552
4	263654	415103

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:06/Issue:11/November-2024 Impact Factor- 8.187 www.irjmets.com

5	260464	416087
6	266455	413070
Mean	263358	414319
Std. Dev.	2486.4	1522.7
%RSD	0.9	0.4

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there was no recognized change in the result and are within range as per ICH Guide lines.

Table 7: Robustness data of Nivolumab and Ipilimumab method.

S.no	Condition	%RSD of NIVOLUMAB	%RSD of IPILIMUMAB
1	F (-) 0.9ml/min	1.0	0.9
2	F (+) 1.1ml/min	0.5	0.8
3	M (-) 55B:45A	1.3	0.7
4	M (+) 65B:35A	0.4	0.2
5	T (-) 25°C	1.3	0.4
6	T (+) 35°C	0.5	0.3

V. DEGRADATION STUDIES

Table 8: Forced degradation conditions for Nivolumab and Ipilimumab

Stress conditions	Solvent	Temperature(C°)	Exposed time
Acid	2N HCl	60°C	30min
Base	2N NaOH	60°C	30min
Oxidation	20% H_2O_2	60°C	30min
Thermal	Diluent	105°C	30min
Photolytic	Diluent	-	1 Day
Hydrolytic	Diluent	60°C	

Table 9: Degradation data of Nivolumab and Ipilimumab

Type of degradation	Nivolumab			Ipilimumab		
	Area	%Recovered	% Degraded	Area	%Recovered	% Degraded
Acid	245567	92.39	7.61	392547	95.07	4.93
Base	246857	92.87	7.13	401857	97.33	2.67
Peroxide	243924	91.77	8.23	404988	98.09	1.91
Thermal	249354	93.81	6.19	408541	98.95	1.05
Uv	259854	97.76	2.24	410578	99.44	0.56
Water	263857	99.27	0.73	411846	99.75	0.25

Assay:

Standard preparations are made from the API and Sample Preparations are from Formulation. Both sample and standards are injected six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. The Average %Assay was calculated and found to be 99.66% and 99.78% for Nivolumab and Ipilimumab respectively.

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:06/Issue:11/November-2024 Impact Factor- 8.187 www.irjmets.com

Table 10: Assy of nivolumab and ipilimumab

	Nivolumab			Ipilimumab		
S.no	Std	Sample	% of Assay	Std	Sample	% of Assay
1	266745	261654	**98.44**	420736	414029	**99.27d**
2	262464	263655	**99.19**	414027	416130	**99.78**
3	266546	268556	**101.04**	412507	418628	**100.37**
4	266574	263656	**99.19**	416221	414117	**99.29**
5	265755	266475	**100.25**	413241	420048	**100.72**
6	263554	265465	**99.87**	420652	413945	**99.25**
Avg	265273	264910	**99.66**	416231	416150	**99.78**
Stdev	1819.8	2441.3	**0.918**	3674.4	2639.3	**0.63**
%RSD	0.7	0.9	**0.9**	0.9	0.6	**0.6**

VI. CONCLUSION

This approach enables quality control laboratories to regularly assess Nivolumab and Ipilimumab, guaranteeing the integrity, consistency, and legitimacy of the product. Medication in bulk and dose forms may be reliably and precisely measured using this method due to its accuracy, specificity, linearity, and precision. With the right mobile phase and detection wavelength in chromatography, the two compounds may be separated and resolved without the need of any excipients or degradation products. In addition to being appropriate for daily analysis, the method satisfies ICH criteria.

VII. REFERENCES

[1] Sharmila Alladi et al., Stability-Indicating HPLC Method for Simultaneous Determination of Drugs Nivolumab And Ipilimumab, Journal of Drug and Alcohol Research (2022) Volume 11, Issue 8

[2] Badikela Rama Krishna et al., A New Stability Indicating Method Development and Validation Report For The Assay Of Nivolumab By RP-UPLC, Journal of Pharmaceutical Negative Results 13(Special Issue 7):1020-1032, November 2022.

[3] D. Dwivedi et al., RP-HPLC Method Development and Validation for Estimation of Nivolumab in Marketed Formulations, Journal of Biomedical and Pharmaceutical Research, November 2022.

[4] K. Sravya et al., Simultaneous Estimation Of The Nivolumab And Relatlimab In Bulk And Pharmaceutical Dosage Form By Using Rp-Hplc, 2023 Ijcrt, Volume 11, Issue 9 September 2023, Issn: 2320-2882

[5] GOPINATH K et al ., A Study Of Method Development, Validation And Forced Degradation For Simultaneous Quantification Of Cabozantinib And Nivolumab In Bulk And Pharmaceutical Dosage Form By Rp-Hplc, Asian J Pharm Clin Res, Vol 12, Issue 2, 2019, 102-106

[6] R. S. Satoskar, S. D. Bhandarkar and S. S. Ainapure. "Pharmacology and Pharmacotherapeutics", 17th edition, Popular Prakashan, Mumbai, India, 2001.

[7] "Burger's Medicinal Chemistry and drug Inertsil", 6 th edition, Wiley Interscience, New Jersey, 2007.

[8] "Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry", 11th edition, Lippincott Williams & Wilkins, New york, 2004.

[9] A. Korolkovas. "Essentials of Medicinal Chemistry", 2nd edition, Wiley Interscience, New Jersey, 1988.

[10] "Goodman and Gilman's The Pharmacological Basis of Therapeutics", 9th edition, McGraw-Hill health professions division, New york, 1996.

[11] Foye's "Principles of Medicinal Chemistry", 6th edition, Lippincott Williams & Wilkins, New york, 2008.

[12] Drugs & Cosmetics Act, 1940 & Rules, 1945, 2nd edition, Susmit publishers, Mumbai, India, 2000.

[13] Indian Pharmacopoeia, Ministry of Health & Family Welfare, Government of India, New Delhi, 1996.