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Research

Analytical method development and validation for the simultaneous estimation of cabozantinib & nivolumab by rp-hplc in bulk and tablet dosage forms.

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Abstract

An accurate, precise, simple, efficient and reproducible, isocratic Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Cabozantinib & Nivolumab in bulk and combined pharmaceutical tablet dosage forms. Cabozantinib and Nivolumab were separated by using a Symmetry ODS C18 (4.6mm×150mm) 5µm Particle Size; Waters Alliance e2695 HPLC system with 2998 PDA detector and the mobile phase contained a mixture of Methanol: 0.1% Orthophosphoric acid (64:36% v/v). The flow rate was set to 1ml/min with the responses measured at 224nm. The retention time of Cabozantinib and Nivolumab was found to be 2.808min and 3.880min respectively with resolution of 5.68. Linearity was established for Cabozantinib and Nivolumab in the range of 20-100µg/ml for Cabozantinib and 60-140µg/ml for Nivolumab with correlation coefficient 0.999. The percentage recovery was found to be is 100.30% for Cabozantinib and 100.21% for Nivolumab respectively. Validation parameters such as specificity, linearity, precision, accuracy and robustness, limit of detection (LOD) and limit of quantitation (LOQ) were evaluated for the method according to the International Conference on Harmonization (ICH) Q2 R1 guidelines. The developed method was successfully applied for the quantification of bulk and active pharmaceutical ingredient present and in combined tablet dosage form.

Keywords: Cabozantinib and Nivolumab, RP-HPLC, Validation, Accuracy, Precision.

INTRODUCTION

HPLC [1-28]

In the modern pharmaceutical industry, high-performance liquid chromatography(HPLC) is the major and integral analytical tool applied in all stages of drug discovery, development and production. It is ideal for the analysis of many drugs in both dosage forms and biological fluids due to its simplicity, high specificity and good sensitivity.

High Performance Liquid Chromatography (HPLC) is a technique that has arisen from the application to liquid chromatography the use of an instrumentation that was originally developed for gas chromatography. High Pressure Liquid Chromatography was developed in the mid-1970 and was improved with the development of column packing material and the additional convenience of on-line detectors. The various components of HPLC are pumps (solvent delivery system), mixing unit, gradient controller and solvent degasser, injector (manual or automatic), guard column, analytical columns, detectors, recorders and/or integrators. Recent models are equipped with computers and software for data acquisition and processing. The mobile phase in HPLC refers to the solvent being continuously applied to the column or stationary phase at a flow rate of 1-5 cm3/min. The mobile phase acts as a carrier for the sample solution. The chemical interactions of the mobile phase and sample with the column determine the degree of migration and separation of components contained in the sample. The mobile phase can be altered in order to manipulate the interactions of the sample and the stationary phase.

Applications of HPLC[2]

a) Natural Products: HPLC is an ideal method for the estimation of various components in plant extracts which resemble in structure and thus demand a specific and very sensitive method e.g., analysis of digitalis, cinchona, liquorice, and ergot extracts.

b) Stability studies: HPLC is now used for ascertaining the stability of various pharmaceuticals. With HPLC the analysis of the various degradation products can be done and thus stability indicating HPLC systems have been developed.

c) Bioassays and its complementation: Complex molecules as antibiotics and peptide hormones are mainly analysed by bioassay which suffer from high cost, necessity replicates, poor precision and length of time required. Also bioassay gives an overall estimate of potency and gives no guidance about the composition. Thus HPLC can be used to complement bioassays and give an activity profile. It has been used for analysis of chloramphenicol, penicillins, clotrimoxazole, sulfas and peptides hormones.

d) HPLC has also been used in the cosmetic industry for quality control of various cosmetics.

Instrumentation 3-8

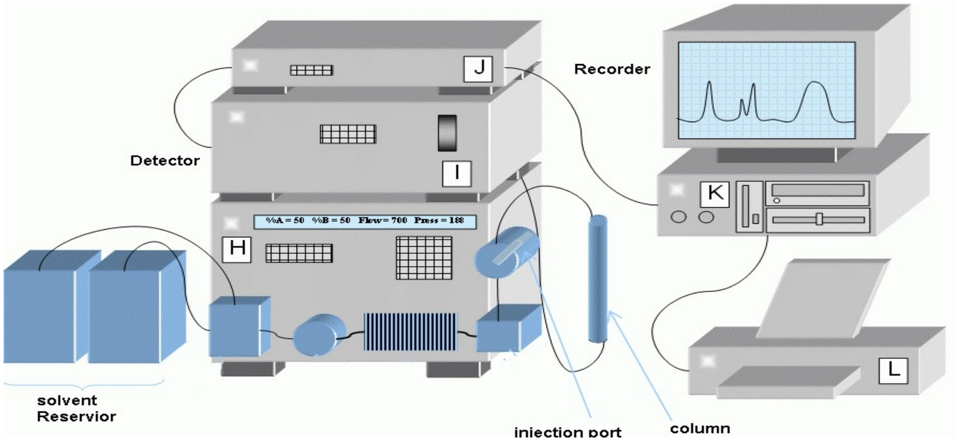

Fig 1: Diagram of HPLC Instrument[3]

MATERIALS AND METHODS

Cabozantinib (Pure)-Suralabs, Nivolumab (Pure)-Suralabs, Water and Methanol for UPLC-LICHROSOLV (MERCK), Acetonitrile for UPLC- Merck, Acetic Acid-Merck.

HPLC method development

Trails

Preparation of standard solution: Accurately weigh and transfer 10 mg of Cabozantinib and Nivolumab working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.6ml of Cabozantinib and 1ml of Nivolumab from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization: Initially the mobile phase tried was Methanol: Water and ACN: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: 0.1% Orthophosphoric acid in proportion 64:36 v/v respectively.

Optimization of Column: The method was performed with various C18columns like Symmetry, X terra and ODS column. Symmetry ODS C18 (4.6mm×150mm) 5µm Particle Size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions

Instrument used : Waters Alliance 2695 HPLC with PDA Detector 996 model.
Temperature : 38ºC
Column : Symmetry ODS C18 (4.6mm×150mm) 5µm Particle Size
Mobile phase : Methanol: 0.1% Orthophosphoric acid (64:36% v/v)
Flow rate : 1ml/min
Wavelength : 224nm
Injection volume : 20µl
Run time : 7.0minutes

Method validation

Preparation of mobile phase

Preparation of mobile phase

Accurately measured 640ml of Acetonitrile (64%) of and 360ml of HPLC Water (36%) were mixed and degassed in a digital ultrasonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Trial 5: (Optimized Condition)

Mobile phase : Methanol: 0.1% Orthophosphoric acid (64:36% v/v)
Column : Symmetry ODS C18 (4.6mm×150mm) 5µm Particle Size
Flow rate : 1 ml/min
Wavelength : 224 nm
Column temp : 38ºC
Sample Temp : Ambient
Injection Volume : 20 µl
Run time : 7 minutes

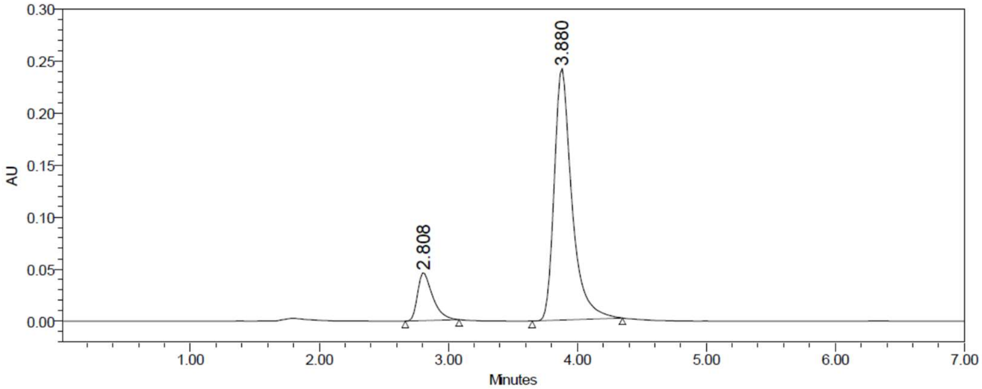

Fig 2: Chromatogram for Trail 5

Table 1: Peak Results for Trail 5

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Cabozantinib	2.808	65259	4327		1.09	5686.4
2	Nivolumab	3.880	8659853	659824	5.69	1.43	6894.7

From the above chromatogram it was observed that the Cabozantinib and Nivolumab peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.
Retention time of Cabozantinib–2.808min
Retention time of Nivolumab – 3.880 min

system suitability

Table 2: Results of system suitability parameters for Cabozantinib and Nivolumab

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Cabozantinib	2.816	65359	4537		1.09	5688.6
2	Nivolumab	3.893	8658747	658986	5.68	1.43	6893.4

- *Resolution between two drugs must be not less than 2.*
- *Theoretical plates must be not less than 2000.*
- *Tailing factor must be not less than 0.9 and not more than 2.*
- *It was found from above data that all the system suitability parameters for developed method were within the limit.*

Assay (Standard)

Table 3: Showing assay standard Results

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Cabozantinib	2.813	65685	4366		1.07	5631.4	1
2	Nivolumab	3.886	8659823	659825	5.68	1.41	6858.2	1
3	Cabozantinib	2.813	65986	4328		1.06	5683.3	2
4	Nivolumab	3.886	8645871	658267	5.67	1.42	6825.1	2
5	Cabozantinib	2.813	65785	4428		1.08	5693.8	3
6	Nivolumab	3.886	8657848	6589413	5.70	1.43	6896.4	3

Assay (Sample)

Table 4: Showing assay sample results

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Cabozantinib	2.799	66858	4459		1.08	5786.4	1
2	Nivolumab	3.863	8756853	669586	5.67	1.42	6957.7	1
3	Cabozantinib	2.799	66259	4463		1.11	5788.5	2
4	Nivolumab	3.861	8769581	663597	5.68	1.44	6944.2	2
5	Cabozantinib	2.799	66436	4439		1.08	5783.1	3
6	Nivolumab	3.863	8754984	668546	5.67	1.45	6928.7	3

Table 5: Assay Results

S.No.	Name of Compound	% Purity
1	Cabozantinib	99.67%
2	Nivolumab	99.47%

The retention time of Cabozantinib and Nivolumab was found to be 2.808mins and 3.880mins respectively. The % purity of Cabozantinib and Nivolumab in pharmaceutical dosage form was found to be 99.67% and 99.47% respectively.

Precision

Table 6: Results of method precision for Cabozantinib

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Cabozantinib	2.808	65899	4366	5683.2	1.08

2	Cabozantinib	2.808	65488	4374	5629.6	1.09
3	Cabozantinib	2.808	65325	4397	5648.7	1.08
4	Cabozantinib	2.808	65983	4329	5637.4	1.09
5	Cabozantinib	2.808	65249	4372	5696.3	1.08
6	Cabozantinib	2.808	65735	4390	5683.7	1.09
Mean			65613.7			
Std. Dev			304.8425			
% RSD			0.464613			

Table 7: Results of method precision for Nivolumab

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Nivolumab	3.880	8659823	658783	6858.4	1.42	5.68
2	Nivolumab	3.880	8658548	657488	6825.6	1.43	5.69
3	Nivolumab	3.880	8659825	652369	6828.3	1.42	5.68
4	Nivolumab	3.880	8659876	658746	6893.7	1.43	5.69
5	Nivolumab	3.880	8658749	658214	6876.2	1.42	5.68
6	Nivolumab	3.880	8659864	652355	6860.8	1.42	5.69
Mean			8659447				
Std. Dev			623.2924				
% RSD			0.007198				

- *%RSD for sample should be NMT 2.*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Intermediate Precision/Ruggedness

Table 8: Results of Intermediate precision for Cabozantinib

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Cabozantinib	2.808	66894	4469	5783.2	1.09
2	Cabozantinib	2.808	66987	4524	5836.1	1.09
3	Cabozantinib	2.808	66259	4476	5863.4	1.10
4	Cabozantinib	2.808	66458	4515	5865.6	1.09
5	Cabozantinib	2.808	66538	4488	5785.9	1.10
6	Cabozantinib	2.808	66299	4566	5749.5	1.10
Mean			66572.5			
Std. Dev			304.536			
% RSD			0.457452			

Table 9: Results of Intermediate precision for Nivolumab

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Nivolumab	3.882	8758569	669584	6983.4	1.43	
2	Nivolumab	3.882	8756983	665985	6936.3	1.44	5.68
3	Nivolumab	3.882	8746926	665346	6985.7	1.44	
4	Nivolumab	3.882	8723655	665324	6953.8	1.43	5.71
5	Nivolumab	3.882	8754983	669853	6899.9	1.44	
6	Nivolumab	3.882	8754699	665875	6977.5	1.43	5.69
Mean			8749302				
Std. Dev			13188.56				
% RSD			0.150738				

- *%RSD of five different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is rugged.*

Accuracy

Table 10: Accuracy (recovery) data for Cabozantinib

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	35922.67	30	30.135	100.447%	100.31%
100%	70895.33	60	60.206	100.342%	
150%	105653.7	90	90.094	100.104%	

- *The % Recovery for each level should be between 98.0 to 102.0%.*

Table 11: Accuracy (recovery) data for Nivolumab

% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	4276303	50	50.207	100.417%	100.22%
100%	8484718	100	100.147	100.149%	
150%	10160608	150	150.092	100.061%	

- *The percentage recovery was found to be within the limit (97-103%).*

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Linearity

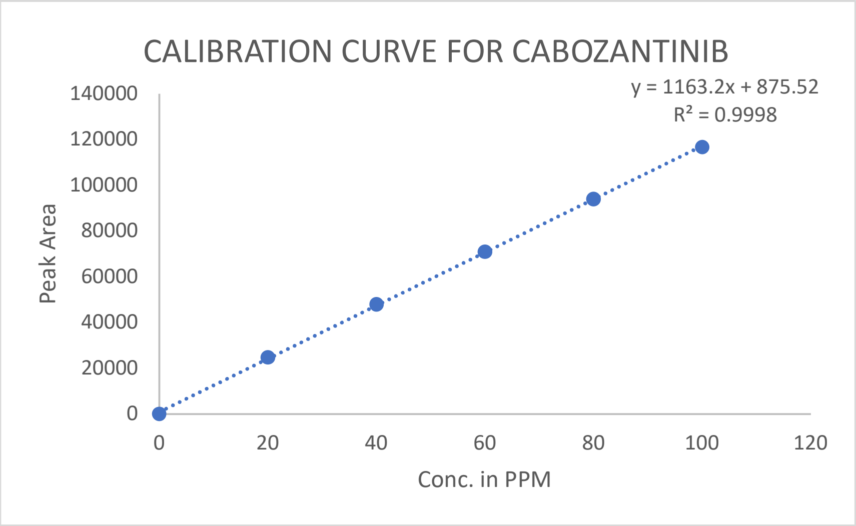

Fig 2: calibration graph for Cabozantinib

Linearity Results: (for Cabozantinib)

S.No	Linearity Level	Concentration (ppm)	Area
1	I	20	24758
2	II	40	47858
3	III	60	70899
4	IV	80	93986
5	V	100	116699
Correlation Coefficient			0.999

Correlation coefficient should be not less than 0.999.

Linearity Results: (for Nivolumab)

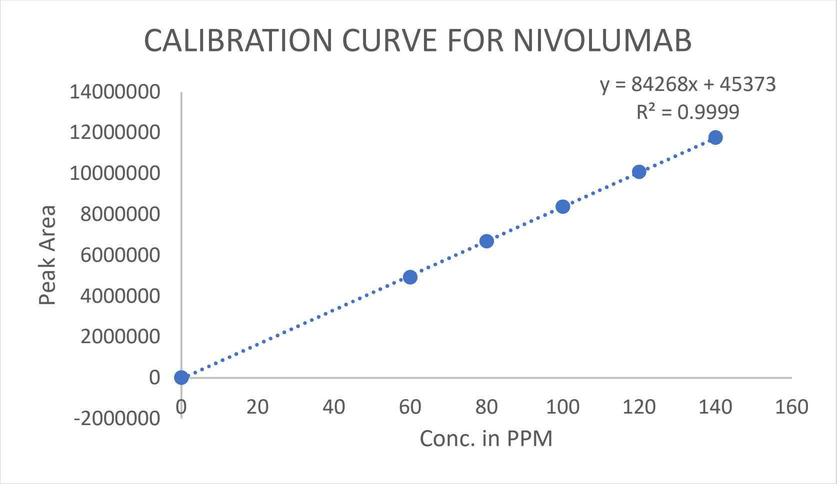

Fig 3: Calibration graph for Nivolumab

S.No	Linearity Level	Concentration(ppm)	Area
1	I	60	4928579
2	II	80	6687843
3	III	100	8389879
4	IV	120	10085848
5	V	140	11769855
	Correlation Coefficient		0.999

Correlation coefficient should be not less than 0.99.

Table 12: Analytical performance parameters of Cabozantinib and Nivolumab

Parameters	Cabozantinib	Nivolumab
Slope (m)	1163	84268
Intercept (c)	875.5	45373
Correlation coefficient (R^2)	0.999	0.999

- *Correlation coefficient (R^2) should not be less than 0.999.*

Robustness

Table 13: System suitability results for Cabozantinib

		System Suitability Results	
.No	Flow Rate (ml/min)	USP Plate Count	SP Tailing
1	0.9	5785.6	1.07
2	1.0	5686.4	1.05
3	1.1	5868.5	1.09

* Results for actual flow (1.0 ml/min) have been considered from Assay standard.

Table 14: System suitability results for Nivolumab

		System Suitability Results	
.No	Flow Rate (ml/min)	USP Plate Count	USP Tailing
1	0.9	6699.3	1.45
2	1.0	6896.7	1.43
3	1.1	6984.6	1.48

** Results for actual flow (1.0ml/min) have been considered from Assay standard.*

Table 15: System suitability results for Cabozantinib

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	5896.3	1.13
2	*Actual	5684.4	1.09
3	10% more	5965.2	1.17

Table 16: System suitability results for Nivolumab

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6786.2	1.47
2	*Actual	6894.7	1.43
3	10% more	6983.4	1.48

CONCLUSION

The study is focused to develop and validate HPLC methods for estimation of Cabozantinib & Nivolumab in bulk and tablet dosage form. For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of samples in a short time period with good robustness, accuracy and precision without any prior separation steps. HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool. The method shows good reproducibility and good recovery. From the specificity studies, it was found that the developed methods were specific for Cabozantinib & Nivolumab.

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