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Research Article

Method development and validation of cabozantinib by LC-MS/MS

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Abstract

The objective of this method is to be simple, precise, and economical performed by LC-MS/MS instrument. The mass spectrometric determination was performed using electrospray ionization in the positive mode with multiple reaction monitoring (MRM) mode and precursor to product ion transition to product ion of m/z 502.2 > 323 for cabozantinib. The effective separation of cabozantinib was achieved X-Bridge (2.1 mm × 100 mm, 3.5 µ) column and the mobile phase composition is 0.2% formic acid: acetonitrile (40:60 v/v), pumped at 0.12 ml/min flow rate. The Rt of cabozantinib was found to be 1.34 minutes. The LOD and LOQ were found at 1.5 ng/ml and 5 ng/ml concentrations and linearity concentrations were in a range of 5 ng/ml to 75 ng/ml with a regression correlation coefficient of 0.999. The % RSD value of accuracy was observed at 1.2–2.0. The marketed formulation assay was found to be 99.82%. The developed method and validation parameters were accepted as per USFDA guidelines.

Keywords

Cabozantinib, LC-MS/MS, Validation, Limit of detection, % RSD

Introduction

Cabozantinib is an anticancer drug and its molecular structure is shown in Fig. 1. The mechanism action of cabozantinib is tyrosine kinase inhibitor (TKI) and effects on vascular permeability factor (VPF) (Yakes FM et al. 2011). A phase-3 randomized controlled study of cabozantinib has higher progression-free-survival (PFS) overall survival (OS) or overall response rate (ORR) as opposed to drug affinitor in patients who progressed following before vascular endothelial factor (VEGF) growing molecularly targeted drugs resulting to its accredited by USFDA (Choueiri TK et al. 2015). Cabozantinib is additionally accepted for utilization in the front line position for patients with midway / low-risk patients (Choueiri TK et al. 2018). Tyrosine kinase (TKs) are considered possible attack for the latest drug progress mostly for cancer and rheumatoid arthritis drugs inhibitors the past various tyrosine kinase inhibitors (TKIs) have been grown and accepted for medicaments of different classifications of cancer with each one targeting certain sign pathways (Nguyen L et al. 2015). Moreover modern further have conducted findings of the janus kinases (JAKs) (Tolaney SM et al. 2016) which by their inhibition established a novel curative path for cancer and immunity disorders (Lacy S et al. 2015).

The heart rhythm problems including long QT intervals observed in inpatient history i.e. the drug should be used with caution (Qaseem A et al. 2012; Takeda H et al. 2017; Osmani L et al. 2018; Van Schil PE et al. 2018; Wienand K et al. 2019; Poole and Jeanne E 2020). Cabo-

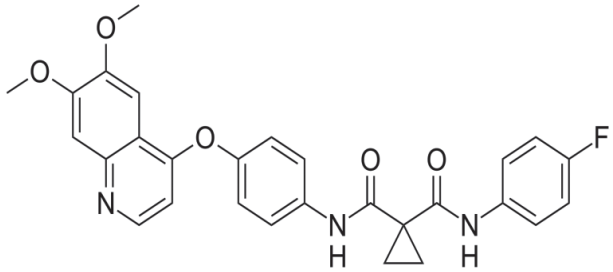

Figure 1. Molecular structure of cabozantinib.

zantinib and nivolumab drugs were marketed under the brand name of opdivo and used for various classifications of cancer treatment include melanoma, lung cancer renal cell cancer, Hodgkin lymphoma head, and neck cancer and colon cancer, and liver cancer. The usual side effects contain tiredness rash, liver problems, muscle pain, and cough (Comi G et al. 2001; Qaseem A et al. 2012; Takeda H et al. 2017; Ashok G and Mondal S 2018; Osmani L et al. 2018; Wienand K et al. 2019).

Materials and methods

Cabozantinib standard powder and API (purity > 98%) were procured from the API industry and marketed tablets procured from the pharmacy store. All HPLC grade solvents were procured from merc india Ltd, india. All chemical reagents and aqueous solvents are purified by using millipore (0.45 μm) filters.

Instrumentation and optimized chromatography conditions

The chromatography analysis was performed by using UPLC instrument waters with an acquity model with an auto sampling system. MS detector is waters Quattro premier XF model trple quadrapole MS was used. The software of the LC-MS/MS system is open lab software. Mass spectroscopy specifications are electrospray ionization (ESI), positive ionization mode, the capillary voltage was set at 3KV, and nitrogen was used as a desolvation gas at a flow rate of 850 L/Hr. The cone voltage is 35 and the cone gas flow is 102 L/Hr.

Separation of the cabozantinib was achieved by the X-Bridge (2.1 × 100 mm, 3.5 μ) column and mobile phase composition of 0.2% formic acid: acetonitrile (40:60 v/v), pumped with 0.12 ml/min flow rate and injection volume is10 μL.

Preparation of standard solution

10 mg of cabozantinib standard pure powder was transferred into 10 ml of volumetric flask and diluted with 10 ml of methanol. This solution concentration is 1000 μg/ml.

Preparation of standard stock solution

0.1 ml of the above standard sample solution was transferred into 10 ml of volumetric flask and diluted with methanol and the resulting solution concentration is 10 μg/ml. This solution was considered a standard stock solution.

Preparation of sample solution

10 mg of cabozantinib active pharmaceutical ingredient powder was transferred into 10 ml of volumetric flask and diluted with 10 ml of methanol. This solution concentration is 1000 μg/ml.

Preparation of sample stock solution

0.1 ml of the above sample solution was transferred into 10 ml of volumetric flask and diluted with methanol and the resulting solution concentration is 10 μg/ml. This solution was considered a sample stock solution.

Method validation

System suitability

The 100% level of cabozantinib standard solution (50ng/ml) was injected 6 times into LC-MS/MS system.

Linearity

The linearity method was determined in the range of LOQ levels (5 ng/ml) to 150% level (75 ng/ml) of cabozantinib samples were injected in the LC-MS/MS system. The regression coefficient value was found from the linearity calibration graph.

Sensitivity (LOD and LOQ)

Limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the following formulas.

$$LOD = 3.3\sigma/s$$

$$LOQ = 10\ \sigma/s$$

Whereas, as σ is the SD of the response (y-intercept) and S is the slope of the linearity plot.

Accuracy

The accuracy method was determined by calculating recovery values at different intervals of LOQ level, 50%, 100%, and 150% level. The % recovery and % RSD values were calculated.

Method precision (repeatability)

The method precision was determined at 100% level (50 ng/ml) of cabozantinib sample 6 replicates were injected and calculated the % RSD.

Intermediate precision

This method was performed by cabozantinib at 100% level (50 ng/ml) of 6 samples injected for different days and calculated the % RSD.

Assay of marketed formulation

Preparation of standard drug solution

10 mg of cabozantinib powder was transferred into 10 ml of a volumetric flask and diluted with methanol. The resulting solution concentration is 1000 µg/ml. Pipette out 0.1ml of the above solution taken into 10 ml volumetric flask and diluted with methanol. The resulting solution concentration is 10 µg/ml. Transferred 0.5 ml of the above solution and dilute with methanol. The resulting concentration is 50 ng/ml and the percentage purity of cabozantinib was calculated.

Preparation of sample drug solution

Weighed 10 tablets and calculated the average weight of the tablet (10.02 mg). Weight equivalent to one tablet of powder was transferred into 10 ml of a volumetric flask and diluted with methanol. The resulting solution concentration is 1000 µg/ml. Pipette out 0.1ml of the above solution taken into 10 ml volumetric flask and diluted with methanol. The resulting solution concentration is 10 µg/ml. Transferred 0.5 ml of the above solution and dilute with methanol. The resulting concentration is 50 ng/ml and the percentage purity of cabozantinib was calculated.

Solution stability

The analyte stability ST% indicates the part of the analyte in a sample that does not degrade before the authentic LC-MS analysis. Prepare 50 ng/ml sample from the stock solution and injected in LC-MS/MS system. The sample solution checks the stability.

Bracketing standard

Bracketing standards are used to analyze the samples, one run before and one after the samples. Prepare 50 ng/ml sample and inject LC-MS/MS system.

Results

MS detection

The predominant protonated precursor [M+H] + ions at m/z 502.27 were obtained from mass spectra of cabozantinib. The detection of ions was determined in MRM mode by transition pairs (precursor to product ion) of m/z 502.13–323.07 for cabozantinib. The molecular ion and product ion is shown in Fig. 2.

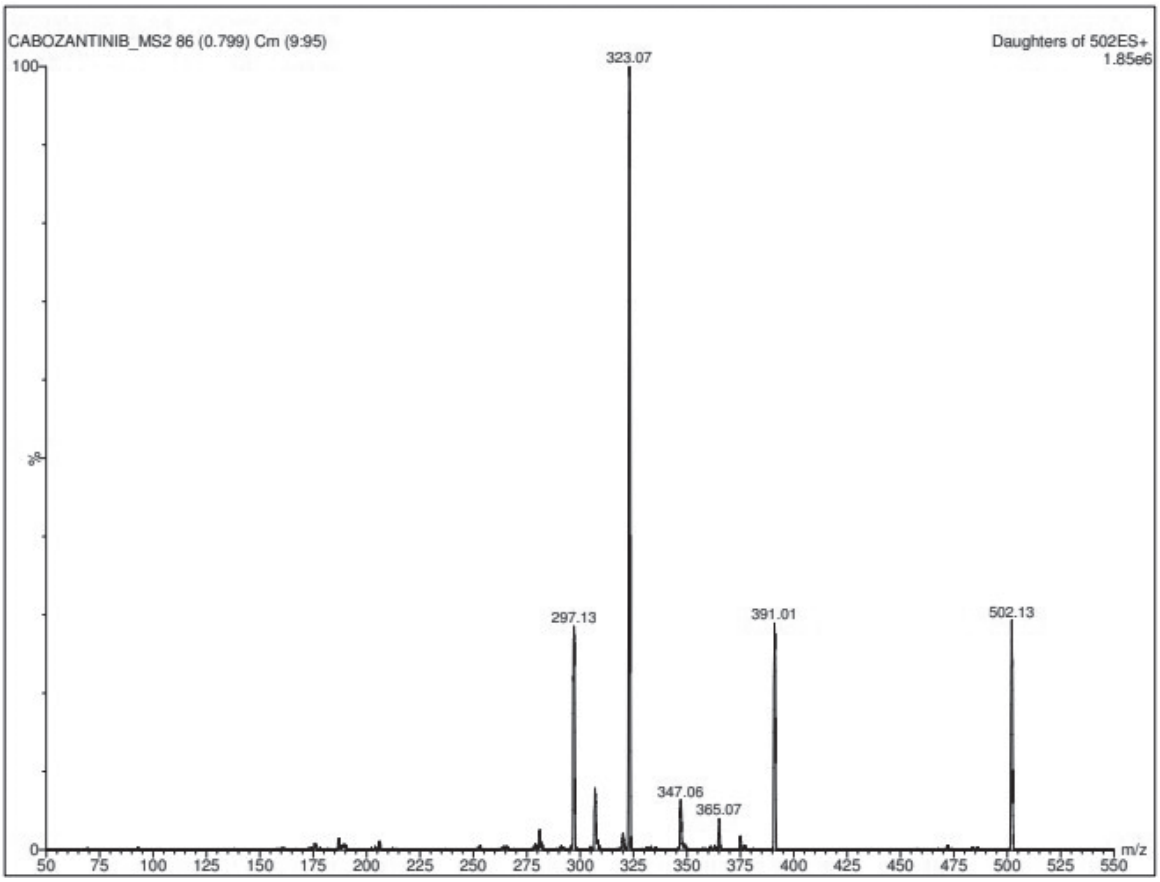

Figure 2. Mass spectra of cabozantinib molecular ion and production.

Optimized method

The cabozantinib method was optimized by using an X-Bridge (2.1 × 100 mm, 3.5µ) column and mobile phase composition of 0.2% formic acid: acetonitrile (40:60 v/v), using 0.12 ml/min flow rate and 10 µL of injection volume, with methanol used as diluents. The retention time was observed at 1.35 min. The optimized chromatogram was given in Fig. 3.

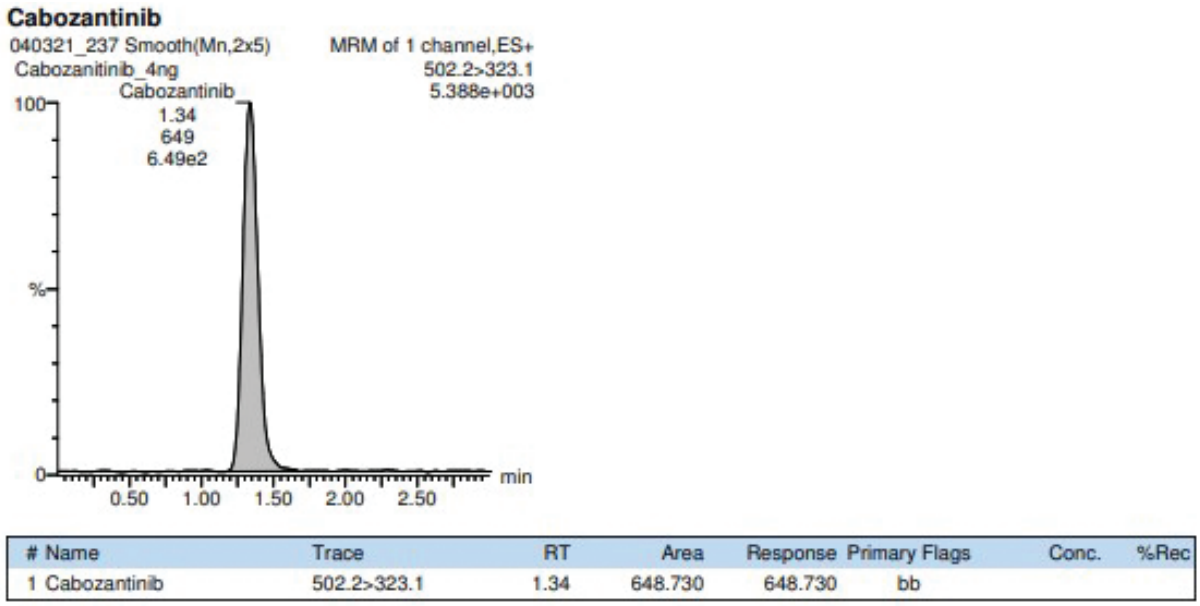

Figure 3. Optimized chromatogram of cabozantinib.

Table 1. System suitability data of cabozantinib.

Injection NO	Peak area
1	3381.354
2	3536.402
3	3452.188
4	3486.185
5	3540.686
6	3550.935
Mean	3491.292
S.D	65.838
% R.S.D	1.88

Table 2. Linearity data of cabozantinib.

S.NO.	Level	Concentration (ng/mL)	Peak area
1	LOQ	5.0	543.432
2	25%	12.5	1067.871
3	50%	25.0	1761.910
4	75%	37.5	2638.744
5	100%	50.0	3257.524
6	125%	62.5	4113.884
7	150%	75.0	4901.912
Linearity equation y = 61.65x+256.0			r^2 = 0.999

Linearity

The regression coefficient (r^2) value is 0.999 obtained from the linearity calibration graph. The linearity graph was given in Fig. 4 and linearity data was given in Table 2.

Method validation

System suitability

The system suitability parameters were evaluated and analyzed to check system performance by using 100% level (50 ng/ml) of the standard solution of cabozantinib. The system suitability % RSD was found to be 1.88. The results data are shown in Table 1.

Sensitivity (LOD and LOQ)

The LOD and LOQ of the cabozantinib 1.5 ng/ml and 5ng/ml of sample concentrations were determined. The LOD and LOQ values are shown in Table 3.

Table 3. LOD and LOQ data of cabozantinib.

Parameter	Concentration (ng/mL)	S/N Ratio
LOD	1.5	5.59
LOQ	5.0	16.94

Accuracy

The accuracy % recovery values were found to be 86.66 – 114.57% and % RSD values were found to be 0.8–2.0%. The accuracy results data was shown in Table 4.

Method precision (Repeatability)

The %RSD values for method precision of the cabozantinib were found to be 1.70% for the 100% level concentration (50 ng/ml).

Intermediate precision

% RSD values of cabozantinib intermediate precision were found to be 1.82%. The method precision and intermediate precision results data were shown in Table 5.

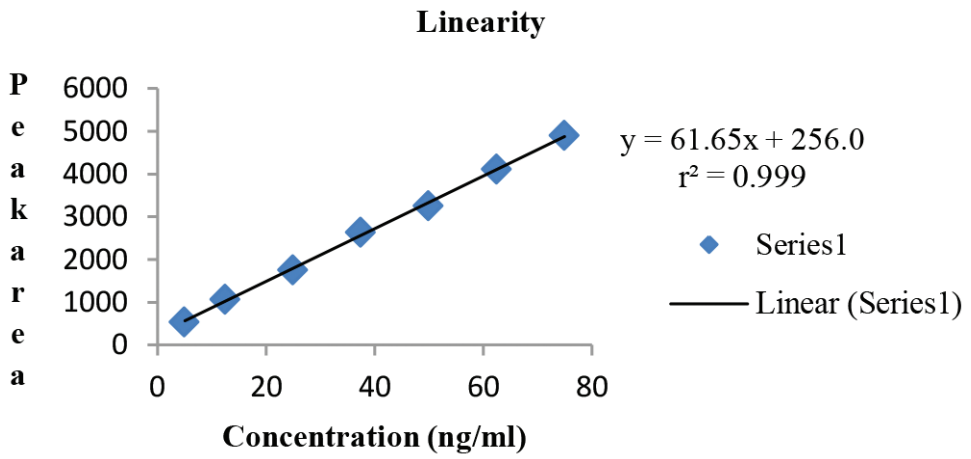

Figure 4. Linearity graph of cabozantinib.

Table 4. Accuracy data of cabozantinib.

Accuracy levels	Concentration (ng/mL)	Peak area	Amount Recovery (ng/mL)	Mean % Recovery ± SD	% RSD
LOQ	5	504.892	4.3	86.66 ± 1.15	1.3
50%	25	504.997	4.3	101.60 ± 2.0	2.0
100%	50	514.299	4.4	106.40 ± 0.872	0.8
150%	75	1807.594	25.1	114.57 ± 1.46	1.2
		1805.662	25.1		
		1863.304	26.0		
		3592.286	53.7		
		3540.686	52.9		
		3550.935	53.0		
		5568.235	85.3		
		5565.929	85.3		
		5687.229	87.2		

Table 5. Method precision and intermediate precision of cabozantinib.

Injection	Concentration (ng/mL)	Method Precision Peak area	Intermediate Precision Peak area
1	50	3627.573	3334.442
2	50	3452.188	3486.185
3	50	3486.185	3478.166
4	50	3540.686	3364.151
5	50	3550.935	3427.706
6	50	3536.402	3381.354
Mean		3532.3281	3412.001
SD		60.093	62.245
% RSD		1.70	1.82

Assay of marketed formulation

The % purity of cabozantinib was found to be 99.82%. The assay results data was shown in Table 6.

Table 6. Assay data of cabozantinib.

S.NO.	Peak area		
	Standard		Sample
1	3486.185	99.82	3478.166
2	3540.686		3536.402
Mean	3513.436		3507.284
SD	38.538		41.179
% RSD	1.09		1.14
% Purity			

Solution stability

The sample solution stability was found to be 99.49%. The solution stability results data was given in Table 7.

Table 7. Solution stability data of cabozantinib.

	Concentration (ng/mL)	Peak area	% Stability
Solution sample	50	3364.151	99.49%
System suitability 1st sample	50	3381.354	

Bracketing standard

Bracketing standard % R.S.D was found to be 1.84%. The bracketing standard results data was given in Table 8.

Table 8. Bracketing standard data of cabozantinib.

S.NO.	Concentration (ng/mL)	Peak area
1	50	3427.706
2	50	3381.354
3	50	3364.151
4	50	3257.524
5	50	3334.442
Mean		3353.035
SD		63.185
% RSD		1.88

Discussion

The cabozantinib is mass detection was performed by positive ionization mode due to the drug's basic nature. The optimization of the chromatogram is achieved by X –bridge column which gives good results. Cabozantinib eluted before 2 min, RT in the existing technique was 1.34 min, and run time which proves it is economical due to the less consumption of mobile phase solvents. Linearity concentration was taken LOQ level and the correlation coefficient of the developed method was very nearest value to 1.0, which supports the sensitivity of the method. This accuracy method % RSD values within limits so that is this method is accurate. Method and intermediate precision were performed which proves that the developed method was precise. The

marketed formulation assay value was found at 99.82%. All the method validation parameters were validated as per USFDA guidelines.

Conclusion

The present research work LC-MS method was successfully developed and validated for the estimation of cabozantinib. This method was economical and precise. The developed method could be practical and reliable to the quality control department of the pharmaceutical industry.

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References

Ashok G, Mondal S (2018) Stability-indicating method development and validation for the estimation of cabozantinib in pharmaceutical dosage forms by ultra-performance liquid chromatography. Asian Journal of Pharmaceutical and Clinical Research 11(10): 238–241. https://doi.org/10.22159/ajpcr.2018.v11i10.27409

Chaudhary AA, Shelke AV, Jadhav AG (2021) Development and Validation of RP-HPLC Method of Cabozantinib in Active Pharmaceutical Ingredient and Pharmaceutical Dosage form. Journal of Pharmaceutical Research International 33(11): 81–90. https://doi.org/10.9734/jpri/2021/v33i1131247

Choueiri TK, Escudier B, Powles T, Mainwaring PN, Rini BI, Donskov F, Hammers H, Hutson TE, Lee JL, Peltola K, Roth BJ, Bjarnason GA, Géczi L, Keam B, Maroto P, Heng DY, Schmidinger M, Kantoff PW, Borgman-Hagey A, Hessel C, Scheffold C, Schwab GM, Tannir NM, Motzer RJ (2015) METEOR Investigators Cabozantinib versus Everolimus in Advanced Renal-Cell Carcinoma. The New England journal of medicine 373(19): 1814–1823. https://doi.org/10.1056/NEJMoa1510016

Choueiri TK, Hessel C, Halabi S, Sanford B, Michaelson M.D, Hahn O, Walsh M, Olencki T, Picus J, Small EJ, Dakhil S, Feldman DR, Mangeshkar M, Scheffold C, George D, Morris MJ (2018) Cabozantinib versus sunitinib as initial therapy for metastatic renal cell carcinoma of intermediate or poor risk (Alliance A031203 CABOSUN randomised trial): Progression-free survival by independent review and overall survival update. Europeain Journal of Cancer 94: 115–125. https://doi.org/10.1016/j.ejca.2018.02.012

Comi G, Leocani L, Rossi P (2001) Colombo B. Physiopathology and treatment of fatigue in multiple sclerosis. Journal of Neurololgy 248(3): 174–179. https://doi.org/10.1007/s004150170222

Inturi S, Avula PR (2018) A sensitive bioanalytical method development and validation of cabozantinib in human plasma by LC-ESI-MS/MS. Brazilian Journal of Pharmaceutical Sciences 54(2): e17163. https://doi.org/10.1590/s2175-97902018000217163

Kadi AA, Abdelhameed AS, Darwish HW, Attwa MW, Bakheit AH (2017) Liquid chromatographic-tandem mass spectrometric assay for simultaneous quantitation of tofacitinib, cabozantinib and afatinib in human plasma and urine. Tropical Journal of Pharmaceutical Research 15: 2683–2692. https://doi.org/10.4314/tjpr.v15i12.21

Kuna AK, Seru G, Radha GV (2019) A novel RP-HPLC method for the quantification of cabozantinib in active pharmaceutical ingredients and pharmaceutical dosage forms. International journal of pharmaceutical sciences review and research 10(8): 3963–69. https://doi.org/10.26452/ijrps.v10i2.263

Lacy S, Hsu B, Miles D, Aftab D, Wang R, Nguyen L (2015) Metabolism and Disposition of Cabozantinib in Healthy Male Volunteers and Pharmacologic Characterization of Its Major Metabolites. Drug Metab Dispos 43(8): 1190–1207. https://doi.org/10.1124/dmd.115.063610

Nguyen L, Holland J, Mamelok R, Laberge MK, Grenier J, Swearingen D, Armas D, Lacy S (2015) Evaluation of the effect of food and gastric pH on the single-dose pharmacokinetics of cabozantinib in healthy adult subjects. Journal of clinical pharmacology 55(11): 1293–302. https://doi.org/10.1002/jcph.526

Osmani L, Askin F, Gabrielson E, Li QK (2018) Current WHO guidelines and the critical role of immunohistochemical markers in the sub classification of non-small cell lung carcinoma (NSCLC): Moving from targeted therapy to immunotherapy. Seminars in Cancer Biology 52(Pt1): 103–109. https://doi.org/10.1016/j.semcancer.2017.11.019

Poole JE (2020) Heart Rhythm O^2: Bringing up-to-date heart rhythm science and information to anyone, anywhere. Heart Rhythm O2 1(1): 1–2. https://doi.org/10.1016/j.hroo.2020.03.005

Pravallika KE, Avula PR (2020) Method Development and Validation for Simultaneous Estimation of Cabozantinib and Nivolumab in Rat Plasma by HPLC. International Journal of Pharmaceutical Sciences Review and Research 61(2): 8–12. https://globalresearchonline.net/journalcontents/v61-2/02.pdf

Qaseem A, Denberg TD, Hopkins Jr RH, Humphrey LL, Levine J, Sweet DE, Shekelle P (2012) Clinical Guidelines Committee of the American College of Physicians. Screening for colorectal cancer: a guidance statement from the American College of Physicians. Annals of internal medicine 156(5): 378–86. https://doi.org/10.7326/0003-4819-156-5-201203060-00010

Rina B Patel, Harsha U Patel, Unnati Patel (2020) Development and validation of stability indicating RP-HPLC method for the estimation of cabozantinib in pharmaceutical dosage form. International Journal of Research and Analytical Reviews 7(4): 892–907. https://doi.org/10.1729/Journal.25170

Satyadev TNVSS (2021) A New Selective Separation method development and Validation of Cabozantinib and Nivolumab Using HPLC. Journal of Pharmaceutical Sciences & Research 13(3): 188–192. https://www.jpsr.pharmainfo.in/Documents/Volumes/vol13issue03/jpsr13032110.pdf

Takeda H, Takai A, Inuzuka T, Marusawa H (2017) Genetic basis of hepatitis virus-associated hepatocellular carcinoma: linkage between infection, inflammation, and tumor genesis. Journal of gastroenterology 52(1): 26–38. https://doi.org/10.1007/s00535-016-1273-2

Tolaney SM, Nechushtan H, Ron IG, Schoffski P, Awada A, Yasenchak CA, Laird AD, OKeeffe B, Shapiro GI, Winer EP (2016) Cabozantinib for metastatic breast carcinoma: results of a phase II placebo-controlled randomized discontinuation study. Breast cancer research and treatment 160(2): 305–312. https://doi.org/10.1007/s10549-016-4001-y

Van Schil PE, Rami-Porta R, Asamura H (2018) The 8th TNM edition for lung cancer: a critical analysis. Annals of Translational Medicine 6(5): e87. https://doi.org/10.21037/atm.2017.06.45

Wienand K, Chapuy B, Stewart C, Dunford AJ, Wu D, Kim J, Kamburov A, Wood TR, Cader FZ, Ducar MD, Thorner AR, Nag A, Heubeck AT, Buonopane MJ, Redd RA, Bojarczuk K, Lawton LN, Armand P, Rodig SJ, Fromm JR, Getz G, Shipp MA (2019) Genomic analyses of flow-sorted Hodgkin Reed-Sternberg cells reveal complementary mechanisms of immune evasion. Blood Advances 3(23): 4065–4080. https://doi.org/10.1182/bloodadvances.2019001012

Yakes FM, Chen J, Tan J, Yamaguchi K, Shi Y, Yu P, Qian F, Chu F, Bentzien F, Cancilla B, Orf J, You A, Laird AD, Engst S, Lee L, Lesch J, Chou YC, Joly AH (2011) Cabozantinib (XL184), a novel MET and VEGFR2 inhibitor, simultaneously suppresses metastasis, angiogenesis, and tumor growth. Molecular cancer therapeutics 10(12): 2298–2308. https://doi.org/10.1158/1535-7163.MCT-11-0264