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A Novel RP-HPLC Method Development and Validation of Mobocertinib in Capsule Dosage Form

Gobalakrishnan P*, V. Munisekhar, V. Hemanth Kumar
Department of Pharmaceutical Analysis, Vagdevi College of Pharmacy and Research Centre, Nellore, A.P, India.

A B S T R A C T

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Mobocertinib, different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm column was preferred because using this column peak shape, resolution and absorbance were good. Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, acetonitrile, dichloromethane, water, 0.1N NaOH, 0.1NHCl). The drug was found to be highly soluble in acetonitrile & dichloromethane and soluble in methanol. Drug was soluble in water. Using these solvents with appropriate composition newer methods can be developed and validated. Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Mobocertinib it is evident that most of the HPLC work can be accomplished in the wavelength range of 200-250 nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 20 µl were found to be the best analysis. The result shows the developed method is yet another suitable method for assay which can help in the analysis of Mobocertinib in different formulations.

Keywords: Mobocertinib, Develosil ODS HG-5 RP C18, Chromatography, HCl, NaOH, RP-HPLC

A R T I C L E I N F O

***Corresponding Author**
Dr. Gobalakrishnan P
Department of Pharmaceutical Analysis,
Vagdevi College of Pharmacy and Research Centre,
Nellore, Andhra Pradesh, India.

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C o n t e n t s

1. Introduction:

Mobocertinib is an oral kinase inhibitor targeted against EGFR and used in the treatment of NSCLC with EGFR exon 20 insertion mutations.

IUPAC Name: propan-2-yl 2-[(4-{[2-(dimethylamino) ethyl](methyl)amino}-2-methoxy-5-(prop-2enamido)phenyl)amino]-4-(1-methyl-1H-indol-3-yl) pyrimidine-5-carboxylate

Chemical formula: $C_{32}H_{39}N_7O_4$
Molecular weight : 585.306 g/mol
Physical Data
Solubility: soluble in DMSO (100 mg/mL (128.86mM)), soluble in water (<1 mg/mL (<1 mM)), ethanol (100 mg/mL (128.86 mM))
Mobocertinib

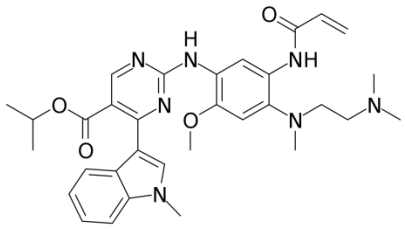

Fig.1

Mechanism of Action:
The epidermal growth factor receptor (EGFR) is a trans membrane receptor that regulates signaling pathways in the control of cellular proliferation. Mutations in these proteins have been associated with certain types of lung cancer, including non-small cell lung cancer (NSCLC). While the majority of EGFR mutations associated with NSCLC involve the EGFR L858R point mutation or exon 19 deletions (referred to as "classical" EGFR mutations), less common EGFR exon 20 insertion mutations carry a particularly poor prognosis and are associated with resistance to standard targeted EGFR inhibitors. Mobocertinib is an inhibitor of EGFR that irreversibly binds to and inhibits EGFR exon 20 insertion mutations at lower concentrations than wild-type EGFR proteins, exerting a pharmacologic effect on mutant variants at concentrations 1.5- to 10-fold lower than on wild-type proteins.
Half-life:
At steady-state, the mean elimination half-life of mobocertinib and its two active metabolites, AP32960 and AP32914, was 18 hours, 24 hours, and 18 hours, respectively.

2. Methodology

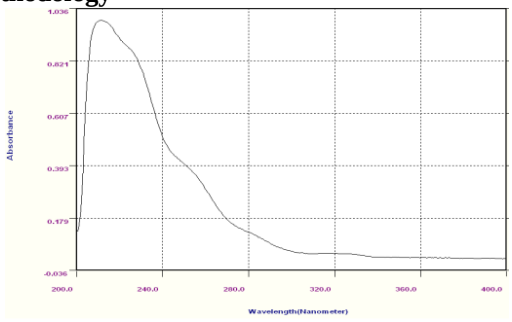

Fig.2:UV spectrum of Mobocertinib

Preparation of standard solution of Mobocertinib
25 mg of Mobocertinib was weighed accurately and transferred into 25 ml volumetric flask. About 10 ml of mobile phase was added and sonicated to dissolve. The volume was made up to the mark with same solvent. The final solution contained about 1000µg/ml of Mobocertinib. From the stock solution again 0.1 ml was taken in a 10 ml volumetric flask & volume was made up to the mark by mobile phase. This solution contains 10µg/ml of Mobocertinib which has been injected to HPLC.
Initialization of the instrument
The HPLC instrument was switched on. The column was washed with HPLC water for 45 minutes. The column was then saturated with mobile phase for 45 minute. The mobile phase was run to find the peaks. After 20 minutes the standard drug solution was injected in HPLC.

Table.1: Chromatographic conditions 1

Mobile phase	Water: ACN(70:30)
Wavelength	223nm
Flow rate	1.0 ml/ min.
Run time	05 min.
Column	waters, C-18, V size (250mm*4.6mmØ)

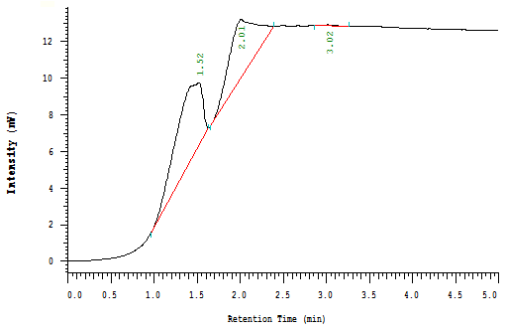

Fig.3: The chromatogram obtained after condition-1

Table.2: Instruments Used

S.no.	Name of Instrument	Instrument Model	Name of manufacturer
1	UV-Visible double beam spectrophotometer	UV 1800	Elico, corp. Japan.
2	HPLC	1575	Hitachi L2130-D2000 Elite
6	Ultra sonicator	--------	Wensar wuc-2L
7	Melting point appraturs	--------	

Table.3: Chemicals Reagents Used

S.no.	Name	Specifications		Manufacturer/ Supplier
		Purity	Grade	
1.	Doubled distilled water	----	----	Sd fine-Chemltd; Mumbai
2.	Methanol	99.9%	A.R.	LobaChem; Mumbai.
3.	Dipotassium hydrogen orthophosphate	96%	L.R.	Sd fine-Chem ltd; Mumbai

4.	Acetonitrile	99.9%	HPLC	LobaChem; Mumbai.
5.	Potassium dihydrogen orthophosphate	99.9	L.R.	Sd fine-Chem ltd; Mumbai
6.	Sodium hydroxide	99.9	L.R.	Sd fine-Chem ltd; Mumbai

3. Results and Discussion

Table 4: Chromatographic conditions 2

Mobile phase	Water: Methanol (30:70)
Wavelength	223nm
Flow rate	0.8 ml/ min.
Run time	05 min.
Column	Hiq Sil, C-18, V size (250mm*4.6mmØ)

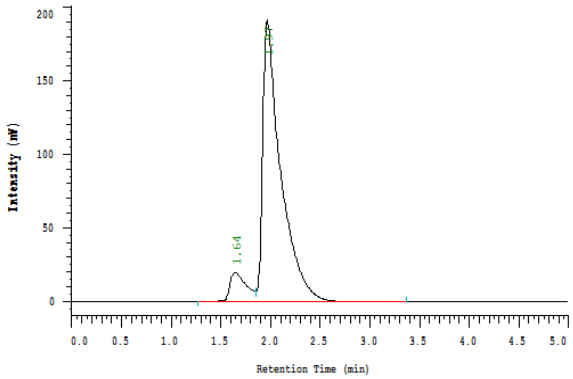

Fig.4: The chromatogram obtained after condition 2

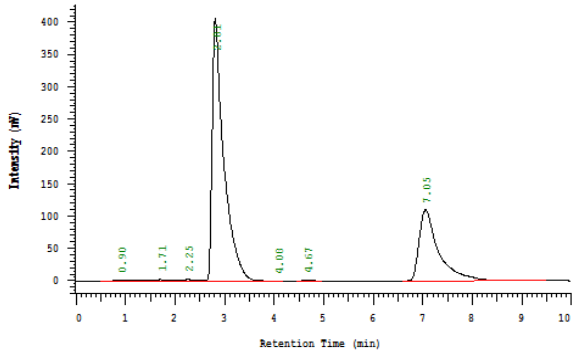

Fig.5: The chromatogram obtained after condition 3

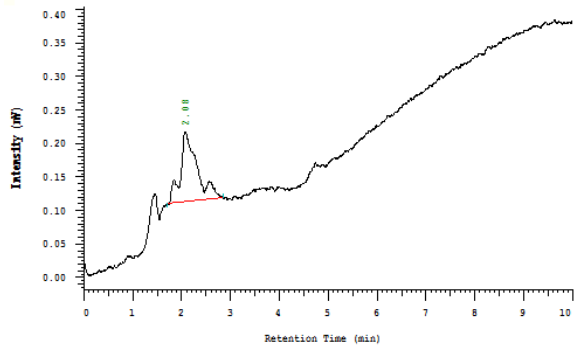

Fig.6: Chromatogram for Blank

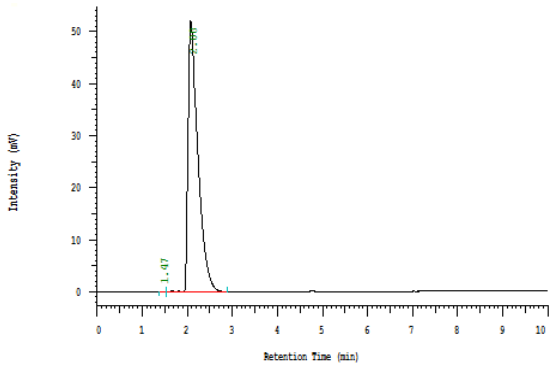

Fig.7: Chromatogram of Mobocertinib

Acid hydrolysis:

An accurately weighed 25 mg. of pure drug was transferred to a clean & dry 25 ml volumetric flask. To which 0.1 N Hydrochloric acid was added & make up to the mark & kept for 24 hrs. from that 4 ml was taken in to a 10 ml volumetric flask & make up to the mark with mobile phase, then injected into the HPLC system against a blank of HCl (after all optimized conditions).

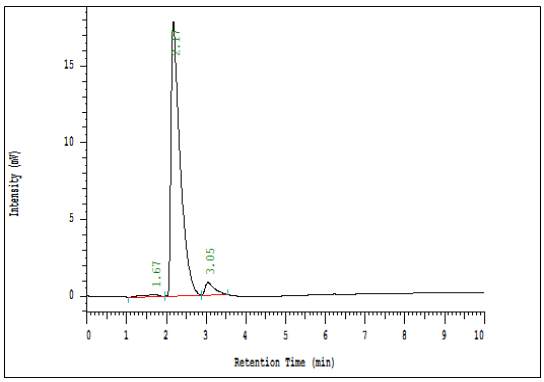

Fig.8: Acid hydrolysis

Basic Hydrolysis:

An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 10 ml volumetric flask. To which 0.1 N Sodium hydroxide was added & make up to the mark & kept for 24 hrs. from that 4s ml was taken in to a 10 ml volumetric flask & make up to the mark with mobile phase, then injected into the HPLC system against a blank of . NaOH (after all optimized conditions)

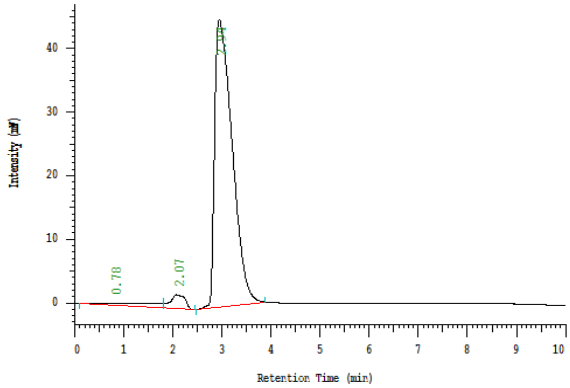

Fig.9: Chromatogram showing degradation related impurity in 0.1 N NaOH

Thermal Degradation: An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 100 ml volumetric flask, make up to the mark with mobile phase & was maintained at 50 ^{0}C. For 24 hrs. Then injected into the HPLC system against a blank of mobile phase.

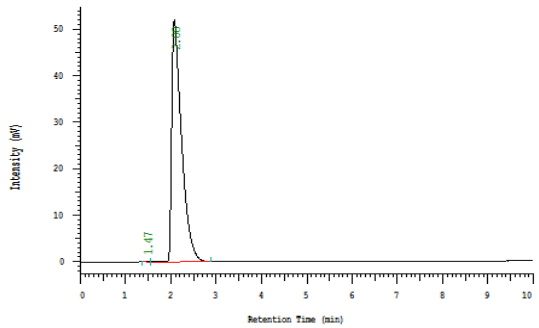

Fig.10: Chromatogram showing thermal degradation studies

Photolytic Degradation: Approximately 10 mg. of pure drug was taken in a clean & dry Petridis. It was kept in a UV cabinet at 254 nm wavelength for 24 hours without interruption. Accurately weighed 1 mg. of the UV exposed drug was transferred to a clean & dry 10 ml.

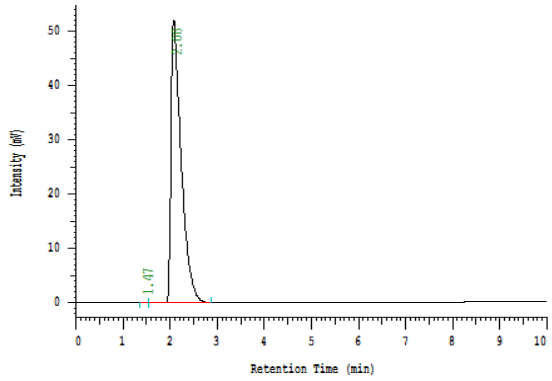

Fig.11: Chromatogram showing photolytic degradation

Oxidation with (3%) H_2O_2: Accurately weighed 10 mg. of pure drug was taken in a clean & dry 100 ml. volumetric flask. 30 ml. of 3% H2O2 and a little methanol was added to it to make it soluble & then kept as such in dark for 24 hours. Final volume was made up to 100 ml. using water to prepare 100 ppm solution.

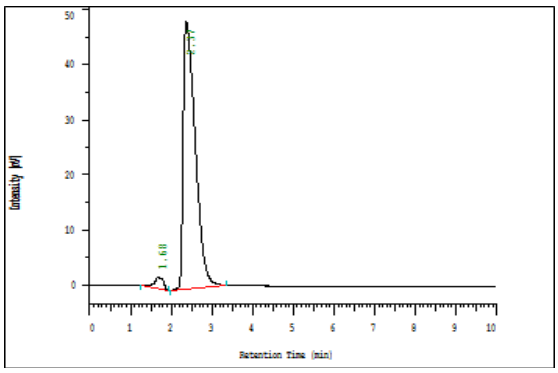

Fig.12: Chromatogram showing oxidative degradation

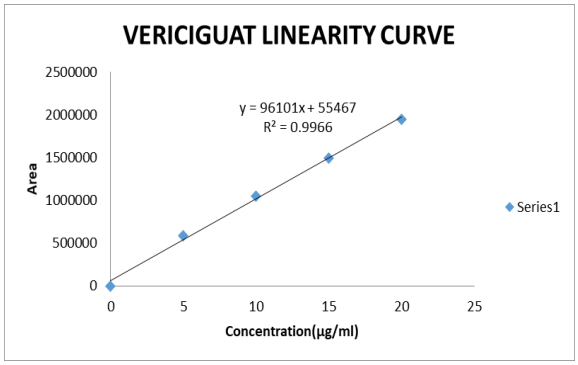

Fig.13: Standard curve for Mobocertinib

Limit of detection and limit of quantification

The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

L.O.D. = 3.3(SD/S).

L.O.Q. = 10(SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

The LOD was found to be 0.452 µg/ml and LOQ was found to be 1.356 µg/ml for Mobocertinib which represents that sensitivity of the method is high.

Table 5: Data of System Suitability Parameter

S.No.	Parameter	Limit	Result
1	Resolution	Rs> 2	9.15
2	Asymmetry	T ≤ 2	Mobocertinib=0.14
3	Theoretical plate	N > 2000	Mobocertinib=3576

Table 6: Results of force degradation studies of Mobocertinib API.

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24Hrs.	40.73	57.35	98.08
Basic Hydrolysis (0.I M NaOH)	24Hrs.	80.93	19.37	100.30
Thermal Degradation (50 ^{0}C)	24Hrs.	99.35	----------	99.35
UV (254nm)	24Hrs.	98.31	---------	99.31
3 % Hydrogen peroxide	24Hrs.	91.37	08.46	99.83

4. Conclusion

A sensitive & selective RP-HPLC method has been developed & validated for the analysis of Mobocertinib API. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. The result shows the developed method is yet another suitable method for assay, purity which can help in the analysis of Mobocertinib in different formulations.

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