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(RP- HPLC) Method Development and Validation of Osimertinib in Tablet Dosage Form

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KEYWORDS
High Performance Liquid Chromatography (HPLC), melanoma, Osimertinib, Acetonitrile, trifluoroacetic acid, Retention time, Acetonitrile, Accuracy, Precision, ICH guidelines, Validation, Method Development.

ABSTRACT:
Osimertinib is widely used for the treatment of melanoma which is type of skin cancer hence, it is essential to develop simple, rapid, sensitive and specific RP-HPLC method for the determination of Osimertinib in tablet dosage form. Osimertinib samples were eluted in isocratic mode by using Supelco (acentis express) C18 column (25cm × 4.6 mm&5 µ) using filtered and degassed mixture of methanol: perchlorate buffer (20:80) %v/v as mobile phase at 45°C with a flow rate of 1ml/min. and injection volume of 20µl and the detection was estimated at 270 nm with the help of UV-VIS detector.The retention time for Osimertinib was observed at 3.28 minutes. The method was linear over the range of 10 to 80 µg/ml with linear regression coefficient (r^2) 0.9994. The RP- HPLC method was validated as per ICH guidelines. The develop method was validated with respect to system suitability, specificity, linearity, precision, accuracy, and robustness. The method was accurate, linear, precise, specific, selective, and rapid suitable for the quantitative estimation of Osimertinib in tablet dosage form. So, the proposed method can be applied for routine analysis of Osimertinib in tablet dosage form.

1. Introduction

Osimertinib is chemically described as N-(2-{2-dimethylaminoethyl-methylamino}-4-methoxy-5-{[4-(1-methylindol-3-yl)pyrimidin-2-yl]amino}phenyl)prop-2-enamide mesylate (fig.1). [1-2].
It is used for the treatment of metastatic Non-small cell lung cancer (NSCLC) in cases where tumour epidermal growth factor receptor (EGFR) expression as detected by FDA-approved testing. Osimertinib is an oral, third-generation EGFR, Tyrosine kinase inhibitor (TKI) drug developed by AstraZeneca Pharmaceuticals. Osimertinib binds to EGFR and also inhibit tyrosine kinase receptor which are responsible for cell growth and cell multiplication. Approximately 10% of patients

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with NSCLC have a rapid and clinically effective response to EGFR-TKIs due to the presence of specific activating EGFR mutations within the tumour cells. [3-8].

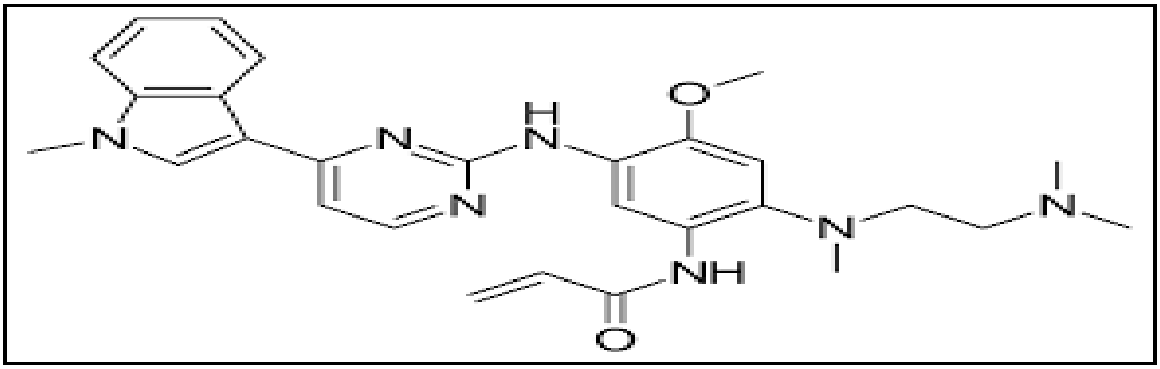

Figure 1:- Structure of Osimertinib

Further the literature survey reveals that one RP- HPLC method was reported for the determination of Osimertinb but the method did not mention properly the composition of the mobile phase and its PH adjuster which was used for that method hence an attempts has been made to develop a new RP-HPLC method which is simple, reproducible for estimation of osimertinib in tablet dosage form by HPLC.

2. Material and Methods

Instrumentation:
Perkin Elmer UV/ VIS Spectrophotometer Lambda 25 connected to Perkin Elmer UV Win Lab software was used for all the spectrophotometric measurements. The analysis was performed on a chromatographic system of Thermo ultra 2000 HPLC using software chromelon with LC instrument control. The HPLC column used was Supelco (acentis express) 25cm × 4.6 mm, 5 µ.

Chemicals and reagents:
Osimertinib working standard was procured from Central Drug Testing Laboratory (CDTL), Mumbai central, Mumbai with claimed potency [99.5 % as is basis]. TAGRISSO (40 mg) Osimertinib tablets were received as gift sample from Assistant Drugs Controller Office, Air Cargo, Mumbai. HPLC Grade methanol from Merck, perchloric acid AR grade from Rankem and Water- Milli-Q Grade were used. All the chemicals were of analytical grade.

Selection of solvent (diluent):
On the basis of solubility and chemical nature of Osimertinib, mixture of methanol and water (50: 50) was selected as diluent for preparation of standard and sample solutions.

Mobile Phase Preparation:
1. Preparation of buffer:
250 ml perchloric acid was added in1000 ml water. PH were adjusted to 3.0 with ortho phosphoric acid and solution was filtered through 0.45 micron membrane filter

2. Preparation of mobile phase: Mobile phase used was perchloric acid and methanol in ratio of (80:20)

Selection of Analytical wavelength:
The 10 µg/mL solution of Osimertinib solution was scanned in the range of 200 – 400 nm. The maximum absorbance of Osimertinib was found at 270 nm.

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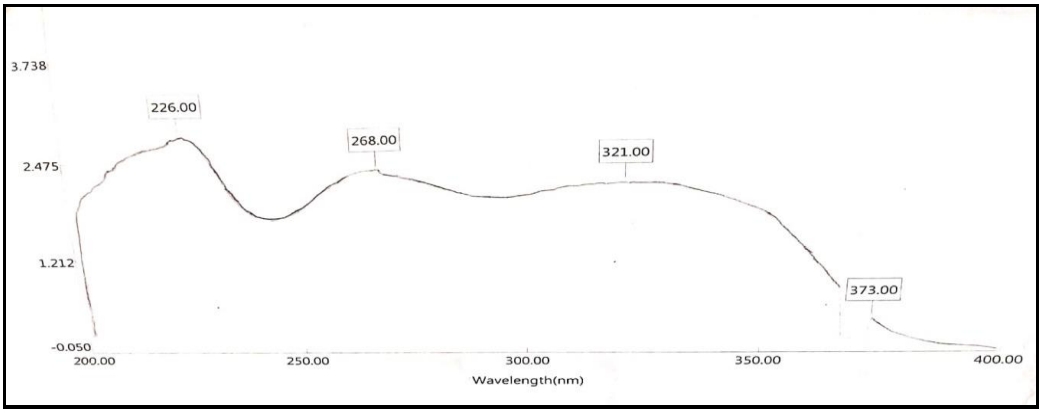

Figure 2:-Osimertinib UV Spectrum

Preparation of standard drug solution:
10 mg of Osimertinib standard was accurately weighed transferred in a 20ml volumetric flask and dissolved by sonication in diluent then volume made with diluent (500 µg/ ml). Then 5 ml from above stock solution was diluted up to 50ml with the diluent (50 µg/ ml).

Preparation of sample solution:
20 Tablets of TAGRISSO (40 mg) were weighed and average weight was calculated. The tablet contents were crushed to fine powder. An accurately weighed quantity of tablet powder equivalent to 40 mg of Osimertinib was dissolved in diluent and sonicated for 15 minutes. Final dilution was made up to100 ml with diluent (400µg/ ml). Then 2.5 ml from above stock solution was diluted up to 20 ml with the same diluent (50 µg/ ml).

Method optimization:
In order to develop an isocratic reversed-phase HPLC method for Osimertinib tablet dosage form, the chromatographic conditions were optimized. Initial trials were started with acetonitrile and buffer with 30:70 ratio using different makes of C18 columns. On Shim-pack GIST C18 column the peak obtained was showing less no. of theoretical plates then next trials was done on Inert sustain column by keeping different ratios of mobile phase but it does not giving stable results. The final method was optimized after some trials by using Supelco (Ascentis Express) C18 column with mobile phase containing methanol and perchloric acid buffer (25mmol) at the ratio of 20:80%v/v. Column oven temperature was kept at 45^0c, flow rate was 1ml/min which gave a good peak shape. At 270nm UV detected wavelength with acceptable System suitability test results.

Validation of Developed Method:
Validation of developed method on HPLC was done as per ICH Q2 (R1) guidelines with respect to various parameters such as system suitability, precision, linearity, accuracy, and robustness. Results of different tests were compared with standard guidelines of ICH Q2 (R1) to get accurate results [9].

Specificity:-
Specificity was evaluated by injecting a blank solution and recording the chromatogram. Peak purity was also established to check for spectral difference, implying that two or more peaks are co-eluting. For Specificity blank, standard drug solution (20µg/ml) and sample solution (20µg/ml) were injected into the HPLC and their chromatogram were recorded. It reveal that the peaks obtained in the standard solution and sample solution at working concentrations are only because of the drugs, as blank and Placebo have no peak at the retention time of Osimertinib. Accordingly it can be concluded that, the method developed is said to be specific.

System Suitability:-
System suitability tests are run to ensure that the instrument can adequately perform intended

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application on a daily basis. This test was performed by injecting 6 replicates of working solution (50 ug/mL) of Osimertinib, and the mean obtained was checked to meet the acceptance criteria of system suitability parameters.

Linearity:
An analytical method's ability to produce linear results with the analyte's concentration in a sample over a specified range is known as linearity. In order to determine the linearity range, six different series of ordinary solutions (10-80ppm.) were examined. The Calibration curve was plotted for Response (Area) vs. Concentration (Amount). The correlation Coefficient (r2) was obtained from the graph.

Precision:
Precision expresses the level of repetition below the very same conditions are unchanged, closeness of value between repetitive injections.
Precision was considered at three levels 50%, 100%, and 150% of working concentration (1, 2, and 3 µg/mL), with a minimum of 6 determinations at each level. Intraday precision was performed on the same day at different time intervals and Interday precision was carried out on two consecutive days.

Accuracy (Standard Addition method):-
Accuracy refers to closeness of the measured value of a quantity corresponds to its "true" value. In that accepted either true value or an accepted reference value and the estimated value. Accuracy shows the closeness of agreement between the value which is received either or traditional true value or an accepted reference value and the value found

Assay:- The assay is tested in specific acceptance criteria set to verify that the results of characteristics and potentially others, depending on the assay and the product of the final method are suitable and reliable for the intended applications.

Robustness:-The evaluation of robustness should be considered during development phase and depends on type of procedure under study robustness show reliability of an analysis with respect to deliberate variation in set method parameters.

3. Results and discussion

Specificity:-
The peak purity of Osimertinib in tablet dosage forms found within the limit which proved that there was no interference of the blank peaks and excipient peaks at the retention time of Osimertinib as shown in figure 3,4 and 5.

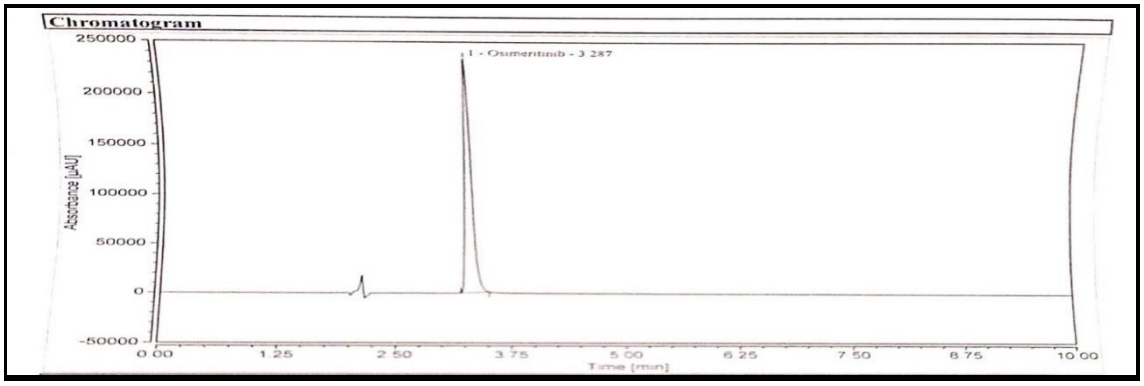

Figure 3:- Chromatogram of standard solution of Osimertinib

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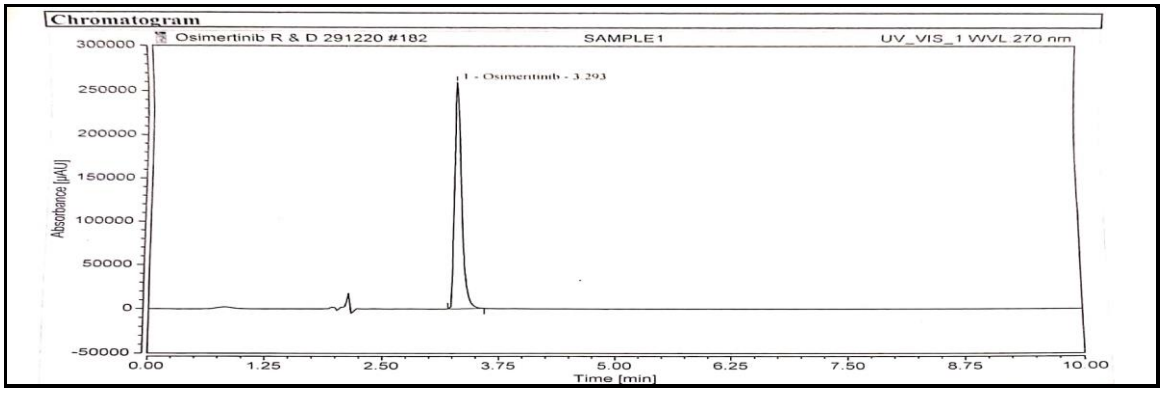

Figure 4:- Chromatogram of sample solution of Osimertinib

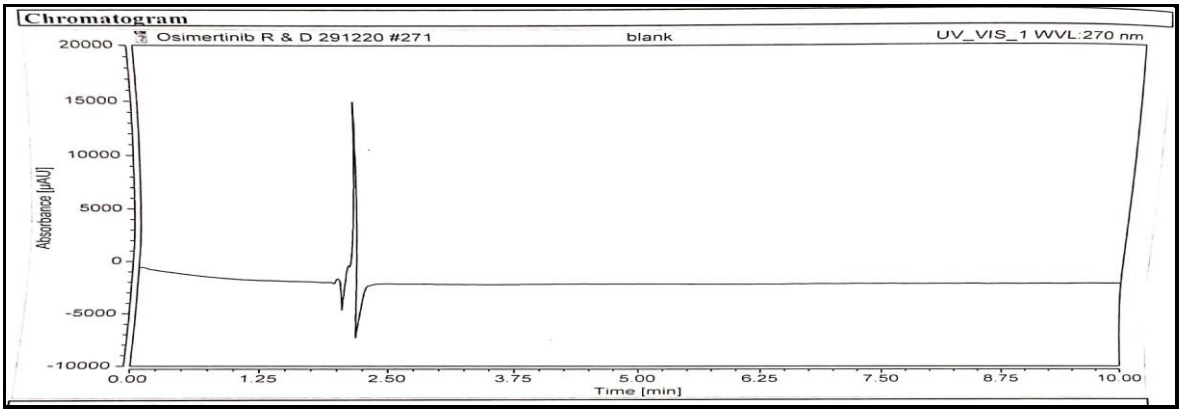

Figure 5:-Chromatogram of blank solution of Osimertinib

System suitability test:

System suitability was done by injecting six replicates injection of standard solution. The peak area, retention time, theoretical plates and tailing factor of standard solution were determined and tabulated in table 1. The obtained results had a standard deviation (S.D.) of not more than 1, and the percentage of relative standard deviation (% RSD) was also not more than 1. Therefore, this method was deemed suitable.

Table 1: System suitability results of Osimertinib

System Suitability				
SR.NO.	Area	Retention time	Tailing factor	Theoretical Plates
1	19890.94	3.27	0.56	10899
2	19881.98	3.27	0.57	10959
3	19868.42	3.27	0.59	11170
4	19867.41	3.273	0.6	11043
5	20006.56	3.27	0.56	11008
6	19890.54	3.27	0.59	11100
MEAN	19900.975	3.2705	0.568333333	11029.83333
SD	52.73524429	0.001224745	0.007527727	97.31889162
%RSD	0.264988244	0.037448246	1.324526662	0.882324226
Limit	NMT 2.0%	NMT 1.0%	NMT 2.0%	NLT 2000

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Linearity:

A 80:20 v/v mixture of perchloric acid and methanol (pH 3) and dilution was made in the range of 10- 80µg/ml for Osimertinib. The calibration graph constructed by plotting concentration of the drug against peak area. A linear correlation in the concentration range of 10-80 µg/ml for Osimertinib was obtained. Calibration curve was shown in Figure 6. The regression equations of this curves was computed and regression coefficient was found to be 0.9994.

Table 2 :-Linearity results of Osimertinib

Linearity level	Concentration (ug/ml)	Average Peak Area
1	10	4120.0
2	20	8060.4
3	30	11827.8
4	50	19953.8
5	60	24023.2
6	80	31185.2

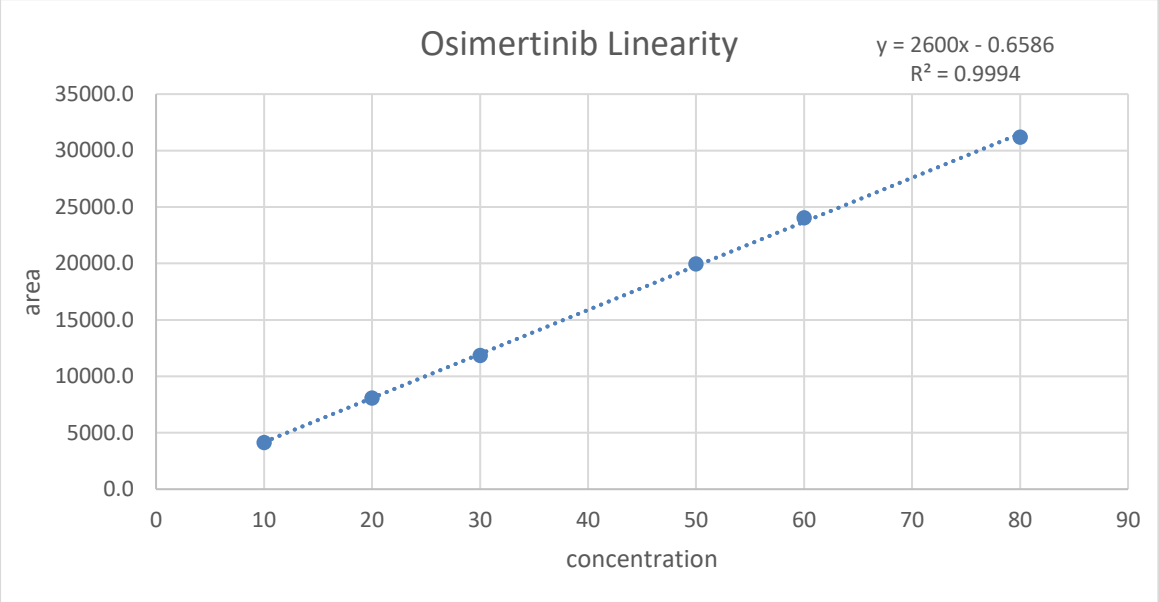

Figure 6:-Linearity Curve of Osimertinib

Precision:

System precision:-

The system precision and method precision performed by injecting six injections of Osimertinib standard and sample the same concentration. Percentage relative standard deviation (%RSD) was calculated from the chromatogram area and it should be less than 2% from precision result for method is precise. The data of system and method precision are tabulated in table 4

Table 3:- System precision of solution (50ppm)

Injection No.	Area at 270 nm
1	19824.29
2	19812.43
3	19788.73

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4	19817.85
5	19833.99
6	19850.83
MEAN	19821.35333
SD	20.94451877
% RSD	0.1056
Limit	NMT 2.0%

Table 4:-Method precision of sample standard solution (50ppm)

Injection No.	Area at 270 nm	%assay
1	20123.36	101.02
2	20094.85	100.87
3	20086.62	100.83
4	20081.49	100.81
5	20153.83	101.17
6	20151.94	101.2
MEAN	20115.3483	
SD	32.4834	
%RSD	0.161	
Limit	NMT 2.0%	

Accuracy (Standard Addition method):-

Accuracy refers to closeness of the measured value of a quantity corresponds to its "true" value. In that accepted either true value or an accepted reference value and the estimated value.

Accuracy of Osimertinib was performed by calculative recovery studies of the test sample at 3 different concentration levels (110%, 120%, 130 %). The mean percentage recovery of Osimertinib was found to be within limit of 98-102% and from percentage recovery results it was found that the developed method is accurate. The percentage recovery results are tabulated in table 5. Estimated % recovery was 99.35%, standard deviation was NMT 1 and % RSD NMT 2% as shown in table 5.

Table 5:-Accuracy results of Osimertinib solution/ percentage recovery Osimertinib

% Level added	STD Spiked (ug/ml)	Amount Recovered (mg)	% Recovery	Mean % Recovery
110	5	43.10091145	98.0	98.01
110	5	43.41508214	98.0	
110	5	43.23301312	98.3	
120	10	47.10293695	98.1	98.13
120	10	47.01723071	98.0	
120	10	47.18741966	98.3	
130	15	53.03328375	102	100.83
130	15	52.9799813	101.9	
130	15	51.26082426	98.6	
			MEAN	99.3533

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			SD	1.4949
	Limit	NMT 2.0%	% RSD	1.4787

Assay:-

For assay of marketed formulation (Tagrisso 40mg). Six tablet were weighed, crushed and equivalent weight was transferred into a 100 ml volumetric flask, sonicated for 10 min. to dissolve and volume was made up with diluent and filtered through 0.45 micron filter then 2.5ml above solution was further diluted into 20ml volumetric flask and percentage assay of marketed formulation was found to be 100.2% shown in table 6.

Table 6:-Assay of Osimertinib

SR No.	Weight of standard (mg)	Sample Weight (equivalent to 40 mg)	Area of standard at 270 nm	Area of sample at 270nm	% Assay
1		1 TABLET		20700	102.0
2		1TABLET		20088.92	101.7
3		1TABLET		20033.99	98.8
4		1TABLET	20185.74	20146.81	99.31
5		1TABLET		20269.55	99.91
6	10	1TABLET		20236.63	99.8
				Mean	100.2
				SD	1.32
	Limit- 2.0%			%RSD	1.318

Robustness:-

The terms robustness and refer to the ability of an analytical method to remain unaffected by small variations in the method parameters (wavelength, temperature, etc.) and influential environmental factors (room temperature, air humidity, etc.) and characterize its reliability during normal usage.

The developed method was evaluated for robustness by small deliberate changes in optimised method parameters such as flow rate (±0.5 ml/min), Temperature (±2°C), Wavelength (±2nm). It was found that none of the above parameters caused by alterations in peak area and retention time. The percentage RSD was found to be within limit and method was found to be robust. The robustness results are shown in table 7.

Table 7:-Robustness of Osimertinib

Parameters	Change in parameter	% Estimation	Mean	SD	% RSD
Wavelength (±2nm)	268	101.3	101.93	0.202	0.2
	270	101.7			
	272	102.8			
Temperature (±2°C)	38	100.9	100.6	0.499	0.497
	40	99.9			
	42	101.0			
Flow rate (±0.5 ml/min)	0.8	101.1	100.86	0.526	0.518
	1	102.0			
	1.2	99.5			

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Limit					NMT 2.0%

4. CONCLUSION

The RP-HPLC method was developed, validated and applied to pharmaceutical analysis for estimation of Osimertinib in tablet dosage form. This HPLC method using common reagent and sample preparation procedure is particularly appropriate for analysis of pharmaceutical dosage form. Study result show that use of a C18 column, the analytes were eluted better, had better resolution, and improved plate count and tailing. Sample recovery shows that recovery of sample as in line with label claim. The developed HPLC method was simple, specific, accurate and precise for Osimertinib in tablet dosage form. It was successfully validated in terms of linearity, accuracy, precision, specificity and robustness in accordance with ICH guidelines. Thus the described method is suitable for routine analysis and quality control of Osimertinib in tablet and bulk dosage forms.

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5. REFERENCES

1. https://go.drugbank.com/drugs/DB09330 accessed on (2nd March 2021)
2. https://pubchem.ncbi.nlm.nih.gov/compound/Osimertinib#section=Information-Sources accessed on (13th March 2021).
3. Osimertinibhttp://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:90943 accessed on (25 th February 2021)
4. Xu M, Xie Y, Ni S, Liu H, The latest therapeutic strategies after resistance to first generation epidermal growth factor receptor tyrosine kinase inhibitors (EGFR TKIs) in patients with non-small cell lung cancer (NSCLC). Ann Transl Med. NCBI 2015, Vol. May;3 (7), pg. no. 96.
5. Tan CS, Gilligan D, Pacey S: Treatment approaches for EGFR-inhibitor-resistant patients with non-small-cell lung cancer. Lancet Oncol. 2015, vol. Sep;16(9), pg. no. 447-59.
6. Talluri Chakradhar and Sumanta Mondal, Goutham Kumar Vanapalli, A new stability indicating RP-HPLC method for estimation of Osimertinib Mesylate, Journal of Drug Delivery & Therapeutics. 2019; vol. 9(2-s), pg. no.391-395.
7. Pramadvara Kallepalli and Mukthinuthalapati Mathrusri Annapurna, A new validated stability indicating UPLC method for the estimation of quantitative estimation of Osimertinib tablets, Acta Scientific Pharmaceutical Sciences 2018, vol. 2, pg. no. 02-06
8. I. Srikanth, A. Prameela Rani, estimation of Osimertinib from human plasma by ESI-LC-MS/MS, Indo American journal of pharmaceutical research 2017,vol. 7(02), pg. no. 7722-7729
9. International Conference on Harmonization (ICH), Q2 (R1) Guidelines for validation of analytical procedures: text and methodology, (Accessed on 15th January,2019).