Available online on 15.04.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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A new stability indicating RP-HPLC method for estimation of Osimertinib Mesylate

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ABSTRACT

A simple stability-indicating high-performance liquid chromatographic method was developed and validated for the determination of Osimertinib Mesylate in tablet dosage forms. Reversed-phase chromatography was performed on Shimadzu Model CBM-20A/20 Alite, using a mixture of 0.1% T.E.A and methanol (50:50, v/v) as mobile phase with a flow rate of 1.0 mL/min. Detection was carried at 211nm. Linearity was observed over the concentration range of 10–400 µg/mL (R^2 = 0.9992) with regression equation y = 125927x + 192648 Osimertinib Mesylate was subjected to stress conditions (acidic, alkaline, oxidation and thermal degradation) and validated as per ICH guidelines. The validated method can be applied to perform long-term and accelerated stability studies of Osimertinib Mesylate formulations.

Keywords: Osimertinib Mesylate; Isocratic elution; Reversed-phase HPLC; Stability-indicating; Validation.

Article Info: Received 28 Feb 2019; Review Completed 05 April 2019; Accepted 09 April 2019; Available online 15 April 2019

Cite this article as:

Chakradhar T, Mondal S, Vanapalli GK, A new stability indicating RP-HPLC method for estimation of Osimertinib Mesylate, Journal of Drug Delivery and Therapeutics. 2019; 9(2-s):391-395 **http://dx.doi.org/10.22270/jddt.v9i2-s.2733**

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1. INTRODUCTION

Osimertinib Mesylate (recently known as mereletinib; exchange name Tagrisso) is a drug used to treat non little cell lung carcinomas with an explicit change. It is a third-age epidermal development factor receptor tyrosine kinase inhibitor. Created by astrazeneca, the prescription was affirmed as a malignancy treatment in 2017 by both the nourishment and medication organization and the European commission.

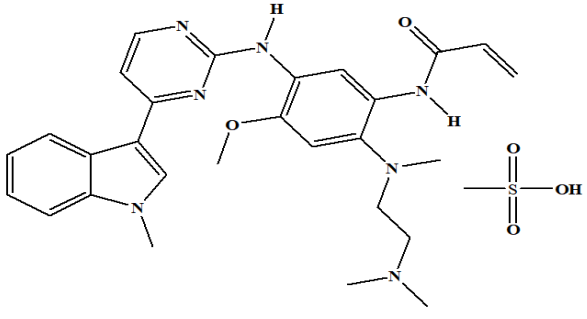


Figure 1: Chemical structure of Osimertinib Mesylate

Osimertinib Mesylate is a little atom tyrosine kinase receptor inhibitor and antineoplastic operator that is utilized in the treatment of chose types of cutting edge non-little cell lung malignant growth (NSCLC). Osimertinib Mesylate is related with a moderate rate of serum aminotransferase rises amid treatment and uncommon cases of clinically obvious intense liver damage.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents:

HPLC grade Methanol (Merck), Tri Ethyl Amine (Merck), sodium acetate trihydrate (Merck), Hydrochloric acid (Rankem), sodium hydroxide (Rankem), Hydrogen peroxide (Rankem) was used. Osimertinib Mesylate, obtained as a gift sample from Sun Pharmaceutical Industries Ltd., (India), was used.

Osimertinib Mesylate is available with brand name Tagrisso (80 mg of the drug per tablet; Wellness Pharma International) were purchased from the local market.

2.2. Instrumentation and conditions:

Chromatographic separation was achieved by using a Shimadzu Mode.000l CBM-20A/20 Alite HPLC system, equipped with SPD M20A prominence photodiode array detector with Phenomenex C8 (250 mm × 4.6 mm i.d., 5 µm particle size) column kept up at 25 ºC.

2.3. Preparation of stock solution:

Osimertinib Mesylate stock solution (1000 µg/mL) was prepared by weighing accurately 10 mg of OSM in a 10 mL volumetric flask with methanol. Working standard solutions were prepared on daily basis from the stock solution with methanol.

2.4. Preparation of 0.1% TEA:

1mL of Tri Ethyl Amine was dissolved in HPLC grade water in a 1000 mL volumetric flask (pH 3.90).

Isocratic elution was performed using 1.0% Tri Ethyl Amine and methanol (50:50, v/v). The overall run time was 10 min. and the flow rate was 1.0mL/min. 20 µL of sample was infused into the HPLC system.

3. METHOD VALIDATION

The method was validated for system suitability, linearity, and limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, selectivity, and robustness.

3.1. Linearity:

Linearity test solutions for the assay method were set up from a stock solution at various concentration levels (10–400 µg/mL) of the assay analyte concentration and 20 µL of each solution was infused into the HPLC system and the obtained peak area region from the chromatogram was noted. The calibration curve was plotted by taking the concentration on the x-axis and the relating peak area on the y-axis. The data were treated with linear regression analysis method. The limit of quantification and limit of detection depended on the standard deviation of the response and the slope of the constructed calibration curve (n=3), as described in ICH guidelines Q2 (R1) [6].

3.2. Precision and accuracy:

The intra-day precision of the assay method was evaluated by carrying out 6 independent assays of a test sample of OSM at three concentration levels (10, 20 and 50 µg mL) (n=3) against a qualified reference standard. The %RSD of three acquired assay values at three diverse concentration levels was determined. The inter-day precision study was performed on three different days i.e. day-I, day-II and day-III at three different concentration levels (10, 20 and 50 µg/mL) and each value is the average of three determinations (n=3). The % RSD of the three obtained assay values on three different days was calculated. The accuracy of the assay method was assessed in triplicate at three concentration levels (50, 100 and 150%), and the percentage recoveries were determined. Standard addition and recovery experiments were conducted to determine the accuracy of the method for the quantification of OSM in the drug product. The study was carried out in triplicate at 50, 100 and 150 µg/mL. The percentage of recovery in each case was calculated.

3.3. Robustness:

The robustness of the assay method was established by providing small changes in the HPLC conditions which included wavelength (206 and 216 nm), the percentage of methanol in the mobile phase (48 and 52%) and flow rate (0.9 and 1.1 mL/min). Robustness of the method was studied utilizing six replicates at a concentration level of 100 µg/mL of OSM.

3.4. Assay of marketed formulations (Tablets):

Solutions were also prepared by extracting the marketed formulations (Tablets) with the methanol and filtered. The filtrate so obtained was diluted as per the requirement and 20 µL solution of each of the marketed formulations (Tagrisso®) was injected into the HPLC system and from the calibration curve, the percentage recovery was calculated.

3.5. Forced degradation studies:

Forced degradation studies were performed to assess the stability indicating properties and specificity of the method. All solutions for stress studies were prepared at an initial concentration of 100µg/mL of OSM and refluxed for 2 hours at 60 ºC and then diluted with methanol.

100µg/mL of OSM solution was exposed to acidic degradation with 0.1 M HCl for 2 hours at 60 ºC the stressed sample was cooled, neutralized and diluted with methanol. Similarly, stress studies were conducted in alkaline conditions with 0.1 M NaOH at 60 ºC for 2 hours and neutralized after cooling with proper dilution with methanol.

Oxidative stress studies were performed using 30 % H2O2 and thermal stress studies were conducted in the thermostat at 60 ºC for 2 hours. 20 µL solution of each of the solutions under forced degradation studies were infused into the HPLC system and the chromatograms were recorded from which the percentage recovery and also the degradants were studied.

4. RESULTS AND DISCUSSION

Initially, the stressed samples were analyzed using a mixture of 0.1% TEA and methanol (65:45, v/v). with a flow rate of 0.9 mL/min in which the resolution and peak symmetry was not satisfactory. The flow rate was changed to 1 mL/min and the drug sample was injected into the loop where a sharp peak was eluted at 3.048 minutes with tailing. Finally, the mobile phase composition was modified as 50:50, v/v and the drug peak eluted were sharp and symmetrical (UV detection at 211 nm) with retention time less than 5 minutes (2.17 ± 0.03 min). Osimertinib Mesylate shows linearity over a concentration range of 0.1–400 µg/mL (Table 1) with % RSD 0.03-0.69. The linear regression equation was found to be y = 145681x + 110238 (R2 = 0.9997). The LOQ was found to be 0.0647µg/mL and the LOD was found to be 0.0931µg/mL. The % RSD range was obtained as 0.24-0.56 and 0.16-0.72 for intra-day and inter-day precision studies respectively (Table 2). 99-100.02 % of recovery was observed in the accuracy studies with % RSD 0.46-0.68 (<2.0 %) (Table 2).

Table 1: Linearity of Osimertinib Mesylate

Conc.	Mean peak area ± SD	%RSD
0.5	517774 ± 551	0.10
1	297878 ± 434	0.14
5	736709 ± 513.5	0.69
10	1469918 ± 4528.5	0.30
20	2718987 ± 2642	0.09
30	4082112 ± 7594.5	0.18
50	6368697 ± 2541	0.03
80	10288450 ± 30482	0.29
100	12905061 ± 82600	0.64

*Mean of three replicates

Table 2: Precision and accuracy studies of Osimertinib Mesylate				
Conc. (µg/mL)	Intra-day precision		Inter-day precision	
	* Mean peak area ± SD (% RSD)		* Mean peak area ± SD (% RSD)	
10	1483496 ± 4747.18 (0.32)		1494269 ± 2390.83 (0.16)	
20	2721896 ± 6532.55 (0.24)		2739617 ± 4931.31 (0.18)	
50	6378120 ± 35717.74 (0.56)		6387120 ± 45987.26 (0.72)	
Accuracy				
Spiked conc. (µg/mL)	Total conc. (µg/mL)	* Mean peak area ± SD (% RSD)	Drug Found (µg/mL)	% Recovery
5 (50 %)	15	2211669 ± 10173.67 (0.46)	14.85	99
10 (100 %)	20	2726354 ± 18539.20 (0.68)	20.04	100.2
15(150 %)	25	3407942 ± 18402.88 (0.54)	24.91	99.64

*Mean of three replicates

The robustness of an analytical procedure refers to its capacity to stay unaffected by small or deliberate variations in method parameters and gives an indication of its reliability for routine analysis. The robustness of the method was evaluated by testing a similar sample under various analytical conditions deliberately changing from the first condition. The outcomes obtained (Table 3) from the assay of the test solutions were not influenced by varying the conditions and were as per the results for unique conditions.

The % RSD value of assay for a similar sample under original conditions and robustness conditions was under 2.0% (0.59-1.23) showing that the developed method was robust.

The representative chromatogram, 3D Curves of Osimertinib Mesylate were shown in Figure 2, 3. The proposed method was applied to the marketed formulations (TAGRISSO®) and the percentage recovery was 100.76-101.61 (Table 4) without the interference from the excipients used in these tablets.

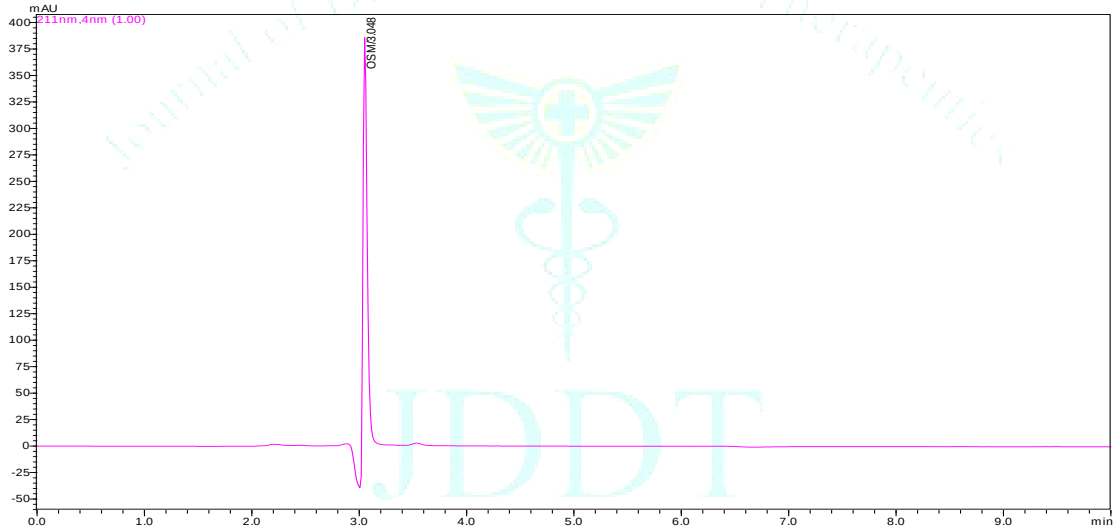


Figure 2: Typical chromatograms of Osimertinib Mesylate Standard Drug (10 µg ml-1).

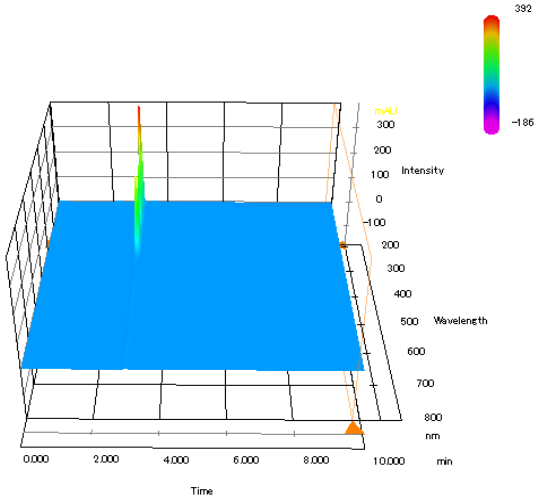


Figure 3: Typical 3D curves of Osimertinib Mesylate (a) Standard Drug (10 µg ml)

Table 3: Robustness study of Osimertinib Mesylate			
Parameter	Condition	*Mean peak area ± SD	(% RSD)
Flow rate (± 1.0 mL/min)	0.9	1433872 ± 17636.62	(1.23)
	1.0		
	1.1		
Detection wavelength (± 5 nm)	206	1483963 ± 8755.38	(0.59)
	211		
	216		
Mobile phase composition (0.1% acetic acid: methanol) (± 2 %, v/v)	48:52	1463789 ± 9368.24	(0.64)
	50:50		
	52:48		

*Mean of three replicates

Table 4: Analysis of Osimertinib Mesylate commercial formulation (Tablets)				
Sample No.	Formulation	Labeled claim (mg)	*Amount found (mg)	*Recovery (%)
1	Brand I	400	393.65	101.61
1	Brand II	800	793.94	100.76

*Mean of three replicates

The stability indicating the capability of the method was established from the separation of OSM peak from the degraded samples. The degradation of OSM was found to be very similar for both the tablets and standard. Typical Chromatogram 3D curves and chromatograms obtained following the assay of stressed samples is shown in Figure 4A-D, 5A-5D, respectively. A slight decomposition was seen on exposure of OSM drug solution to acidic (0.62%), alkaline (1.76%), oxidative (7.37%) and thermal (1.83%) conditions. The degradants were observed at 3.099, 2.944, 2.763 and 2.795 min were observed. The percentage of drug degradation was less than 10% in all stressed conditions indicating that Osimertinib Mesylate is very much resistant (Table 5).

The present stability-indicating method for the determination of OSM in pharmaceutical formulations is specific because the drug peak was well separated even in the presence of degradation products. Also, Osimertinib Mesylate is more resistant towards all degradations and the overall data demonstrated that the excipients and the degradation products did not interfere with the OSM peak. The system suitability parameters for the OSM peak shows that the theoretical plates were more than 2000 and the tailing factor was less than 2 (Table 5).

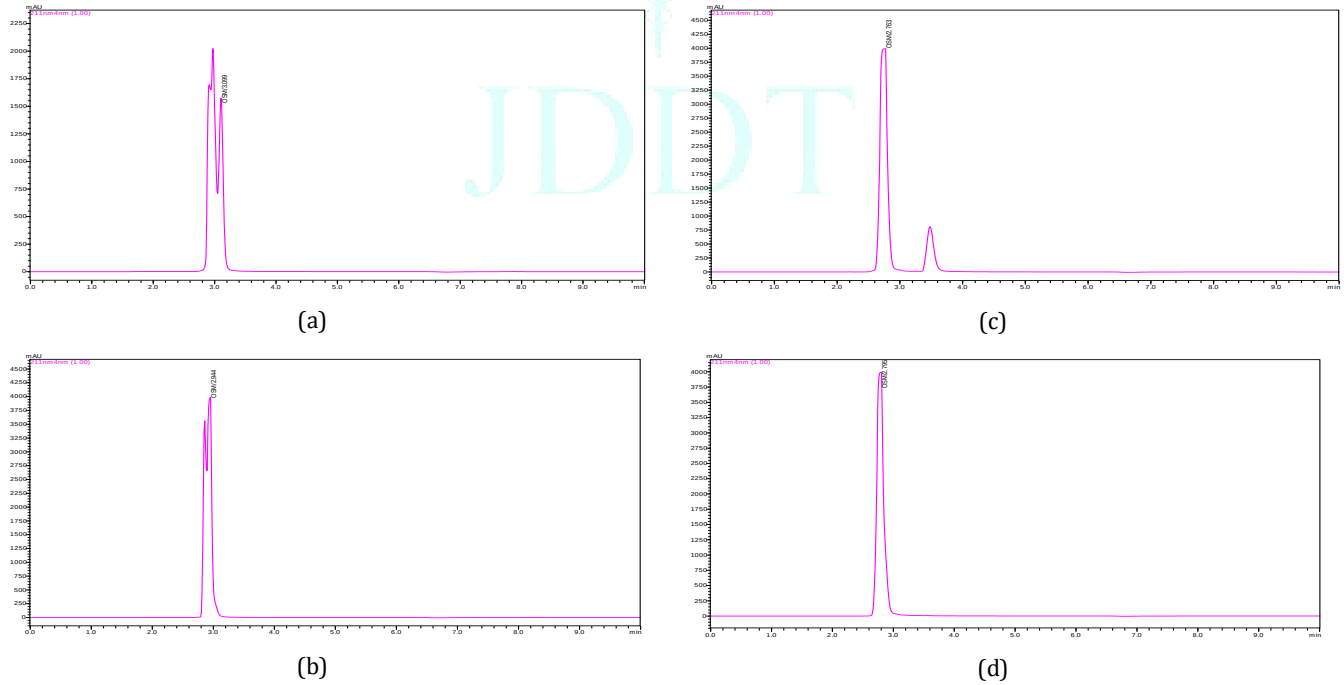
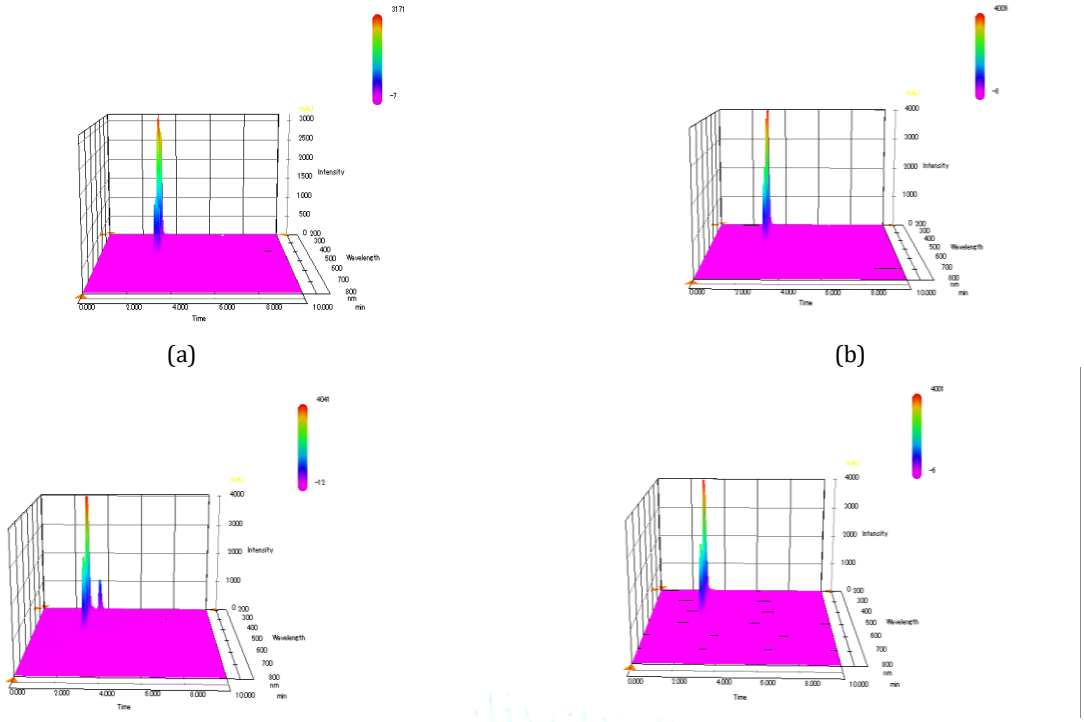


Figure 4: Typical Chromatograms of Osimertinib Mesylate (10µg/ml) on (a) Acidic degradation, (b) Alkaline degradation, (c) Oxidative degradation and (d) Thermal degradation.



(a) (b)

(c) (d)

Figure 5: Typical 3D Curves of Osimertinib Mesylate (10µg/ml) on (a) Acidic degradation, (b) Alkaline degradation, (c) Oxidative degradation and (d) Thermal degradation.

Table 5: Forced degradation studies of Osimertinib Mesylate

Stress Conditions	*Retention time	*Drug recovered (%)	*Drug decomposed (%)	Theoretical plates	Tailing factor
Standard drug (Untreated)	3.048	100	-	7548.15	1.320
Acidic degradation 1 ml 0.1N HCl, 80°C, 30 mins	3.099	99.38	0.62	11110.044	1.310
Alkaline degradation 0.1 ml 0.1N NaOH, 80°C, 30 mins	2.944	98.24	1.76	9231.985	1.319
Oxidative degradation 1 ml 3% H_2O_2, 80°C, 30 mins	2.763	92.63	7.37	2764.708	0.905
Thermal degradation 105°C, 30 mins	2.795	98.17	1.83	3108.060	1.289

*Mean of three replicates

5. CONCLUSION

The proposed stability-indicating HPLC method was validated as per ICH guidelines and applied for the determination of Osimertinib Mesylate in pharmaceutical dosage forms and can be successfully applied to perform long-term and accelerated stability studies of Osimertinib Mesylate formulations.

ACKNOWLEDGEMENT

We are grateful to M/s GITAM (Deemed to be University), Visakhapatnam, India for providing research facilities and MSN India Private Limited (India), India for providing the gift samples of Osimertinib Mesylate.

CONFLICT OF INTEREST

There is no conflict of interest between authors.

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