Bangladesh Pharmaceutical Journal ***27****(1): 85-91, 2024 (January)*

Development and Validation of RP-HPLC Method for Routine Analysis of Osimertinib

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(Received: November 15, 2023; Accepted: January 7, 2024; Published (web): January 25, 2024)

Abstract

Osimertinib is a newly approved treatment line for the non-small cell lung carcinoma actively caused by mutation in EGFR and T790M. The present study was targeted to originate a validated method for the qualification as well as quantification of osimertinib through RP-HPLC in bulk and its pharmaceutical formulation. The mobile phase, 65% triethylamine buffer (pH 2.5) and 35% acetonitrile (v/v) was run through a prontosil column (C18, 4.6×150 mm i.d., 3.5 µm particle size) at a flow rate of 0.7 ml/min. A PDA plus detector was used to scan the absorbance over 210 to 360 nm. Validation study performed following the ICH guidelines Q2 (R1) was found the developed method to be specific, precise, accurate, linear over the range of 64–96 µg/ml with $R^2 > 0.999$, robust and rugged. The % recovery of osimertinib at different levels ranged between 99.32% and 100.37% from tablet formulation (Osicent). The method holds promise for analysis of osimertinib in bulk, pharmaceutical formulations and for further research.

Key words: Osimertinib, RP-UHPLC, C18, ICH, Validation.

Introduction

Between the two most common forms of lung cancer, the non-small cell lung cancer (NSCLC) holds 5.6 times more prevalence than small cell lung cancer (Herbst *et al.,* 2008). Mutation in the epidermal growth factor receptor (EGFR) is an important marker for developing NSCLC (Herbst *et al.,* 2008; Ferlay *et al.,* 2015). But treating NSCLC patients has become an arduous task due to the emergence of a secondary mutation in exon 20 that is to say T790M resistance (Metro and Crinò, 2012; Denis *et al.,* 2015). Due to the development of T790M mutation resistance, the current therapeutic regime has become ineffective in treating NSCLC which urged the development of next generation therapy (Wang *et al.,* 2016; Soejima *et al.,* 2017).

Osimertinib (OSIM), a small molecule chemically belonging to mono-anilino-pyrimidine with molecular formula of $C_{28}H_{33}N_7O_2$ is a third-generation, irreversible tyrosine kinase inhibitor (TKI) (Butterworth *et al.,* 2017; Soria *et al.,* 2018; Zhu *et al.,* 2017). It acts by binding T790M resistant mutations and EGFR activating mutations irreversibly, leaving wild-type EGFR unaffected (Wang *et al.,* 2016; Zhang *et al.,* 2016). Owing to better outcomes than other tyrosine kinase inhibitors acting on epidermal growth factor receptors, osimertinib is now approved for use as both first- and second-line therapy (Mok *et al.,* 2017).

During product development, the analytical method holds paramount importance (Shill *et al.,*

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DOI: https://doi.org/10.3329/bpj.v27i1.71158

2022). To date, several chromatographic methods associated with mass spectrometry have been reported which highlighted analysis of osimertinib or contemporaneous analysis of multiple EGFR-TKIs in human plasma, serum, whole blood, cerebrospinal fluid or urine (Rood *et al.*, 2016; Xiong *et al.*, 2017; Irie *et al.*, 2019; Rood *et al.*, 2020; Mitchell *et al.*, 2019; van Veelen *et al.*, 2020; Ma *et al.*, 2021; de Leeuw *et al.*, 2023). In this current study, the aim was to develop a non-cumbersome, rapid, robust and validated RP-HPLC for routine analysis of osimertinib in pharmaceutical formulations as well as in bulk drug.

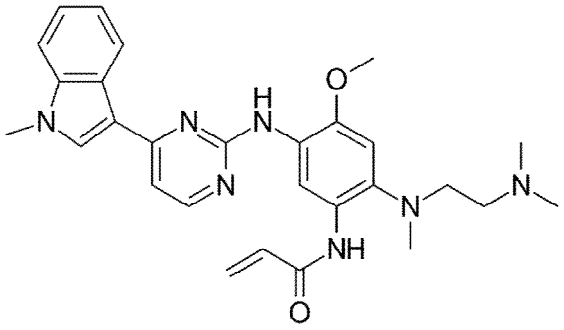

Figure 1. Structure of Osimertinib.

Materials and Methods

Chemicals: Osimertinib drug powder was obtained as a gracious patron- Incepta Pharmaceuticals Ltd., Bangladesh, while the osimertinib tablet (Osicent) was purchased from the local market in Dhaka, Bangladesh. HPLC grade acetonitrile was of RCI Labscan Ltd., Thailand. Triethyl amine, and methanol were purchased from BDH, England. Phosphoric acid was obtained from Merck, Germany. The water utilized in the experimental procedures was purified by the LaboStar® reverse osmosis deionization system manufactured by Evoqua (Germany).

Liquid chromatographic conditions: The Flexar series UHPLC from Perkin Elmer was used consisting of FX-15 binary pump, vacuum degasser, an auto-sampler, column oven, and PDAplus detector. The chromatograms and the data were obtained by linking a Chromera manager (version 4.0) software to computer. A Prontosil C18 column (150 x 4.6 mm i.d., 3.5 µm particle size) was used for the chromatographic separation. A fixed injection volume of 15 µL and a column temperature of 30^{o}C were employed. The chromatograms were processed at 283 nm due to the highest absorbance. Triethylamine buffer (pH 2.5) and acetonitrile in a ratio of 65:35 (%v/v) was used as the mobile phase, where, the flow rate was set at 0.7 ml/min. The total run time was 10 minutes.

Preparation of reagents: To prepare 1M phosphoric acid (H_3PO_4) solution, 5 ml of 98% phosphoric acid was added drop by drop to 20 ml of distilled water. This was then diluted with distilled water to make a final volume of 50 ml. For 1M ammonium hydroxide (NH_4OH) solution, 1.75 g of ammonium hydroxide was dissolved in 20 ml of distilled water in a volumetric flask, followed by a distilled water dilution to 50 ml. The triethylamine buffer (pH 2.5) was prepared by combining 10 ml of triethylamine with 1000 ml of water and adjusted it with phosphoric acid or ammonia solution.

Mobile phase preparation: The triethylamine buffer and acetonitrile were mixed in a 65:35, %v/v ratio. The mixture was subjected to sonication for a minimum of 15 minutes. The sonicated mobile phase was filtered using a vacuum filter from RESTEK fitted with a 0.22 µm membrane filter.

Standard solution preparation: A precisely weighed amount of 100 mg of osimertinib was taken to a volumetric flask (100 ml) and dissolved in 30 ml diluent, which was the same as the mobile phase. Finally, a concentration of 1 mg/ml was achieved by adding diluent up to the mark. The resulting solution was sonicated for 10 minutes. To meet the experimental requirements, five different concentrated solutions (64 µg/ml, 70 µg/ml, 80 µg/ml, 90 µg/ml, 96 µg/ml) were prepared by diluting the osimertinib solution with the diluent (mobile phase).

Sample solution preparation: Average weight of five osimertinib tablets were calculated. They were crushed and coarsely pulverized. Equivalent powdered sample to 50 mg of osimertinib was taken

to a 50 ml volumetric flask, mixed with 20 ml of diluent, and sonicated for 20 minutes to ensure complete drug dissolution. After cooling to ambient temperature, the volume was adjusted with the same diluent. Whattman filter paper (No. 42) was used to filter this solution. From this filtrate, the required concentrations of sample solutions (64 µg/ml, 70 µg/ml, 80 µg/ml, 90 µg/ml, 96 µg/ml) were prepared using the diluent.

Method validation

The validation of the formulated approach adhered to the guidelines outlined in ICH Q2 (R1) and is expounded upon as follows.

System suitability: System suitability of the proposed method was evaluated by injecting osimertinib working standards (80 µg/ml) using an auto sampler. Repeatability, theoretical plates, tailing factor, and retention time were recorded for six replicates.

Linearity: The linearity was determined by evaluating five osimertinib working solutions in triplicate spanning the concentration range 64 to 96 µg/ml, which corresponds to 80 to 120 percent of the nominal test concentration (80 µg/ml) for regular osimertinib analysis. The concentrations of 64, 70, 80, 90, and 96 µg/ml were used to create the calibration curve. R^2 value was employed to assess the linearity of the data.

Specificity: Placebo testing used to determine the specificity of the established approach. For this investigation, a placebo of osimertinib tablet was created (formulations comprising all of the formulation ingredients except osimertinib) and handled similarly the sample was tested.

Accuracy: Recovery experiments were used to assess the proposed method's accuracy. The normal addition approach ensures it. Both of the results were compared to what was expected. The procedure is repeatable if the relative standard deviation (RSD) of all parameters is less than 2%.

Precision: Both the standard (80 µg/ml) and sample (80 µg/ml) osimertinib solutions were analyzed in six repetitions on the same day (intra-day precision) and six times a day for three days (inter-day precision). The percent RSD derived from the obtained data represents the precision of this method.

Sensitivity: Analyzing the sensitivity of the analytical method involves establishing the limits of detection (LOD) and quantitation (LOQ). The process includes injecting a blank sample into the HPLC system, monitoring pump pressure fluctuations. Very diluted standard osimertinib solutions are then introduced until minimal pressure fluctuation is achieved. According to ICH guidelines, LOD and LOQ are calculated using the signal to noise ratio. The ratio of 3:1 was used as LOD value and 10:1 as LOQ.

Ruggedness: Ruggedness assessment involved the independent analysis of six replicate solutions of osimertinib tablet formulation at a concentration of 80 µg/ml by two distinct analysts. This scrutiny aimed to verify the reproducibility of test results. In both instances, computations were performed for percentage recovery and percent relative standard deviation (% RSD).

Robustness: The resilience of the current methodology to minor, deliberate variations in parameters, termed robustness, was examined. Adjustments to buffer solution pH, mobile phase composition, and flow rate were made to assess the method's stability.

Results and Discussion

Development of the method: Prontosil C-18 column (150 mm x 4.6 mm i.d, 3.5 micrometer sized particle) with acetonitrile (35% v/v) and triethylamine buffer (65%, pH 2.5 ± 0.2) as the mobile phase is used for the analysis of osimertinib in this method.

The chromatograms exhibited no interference with blank. Therefore, the developed method was subjected to validation study.

Validation of the developed method: Results found for the validation parameters according to ICH Q2(R1) are mentioned below.

System suitability: System suitability of the method was studied using six replicates of osimertinib standard solution at concentration of 80 µg/ml. %RSD values determined for peak area, tailing factor, theoretical plate count and retention time were found within acceptable limits (Table 1).

Table 1. System suitability parameters (n=6).

Parameters	Value (Mean ± % RSD)	Acceptable limit
Peak Area	5076883.83± 0.04	% RSD ≤ 1
Tailing Factor	1.149 ± 0.71	≤ 2.0
Theoretical Plate	5703.53 ± 0.37	> 2000
Retention Time	3.640 ± 0.28	% RSD ≤ 0.5

Specificity: Chromatogram attained for osimertinib and related compounds demonstrated high resolution under several conditions without any interference of retention time of blank (Figure 2a, 2b & 2c).

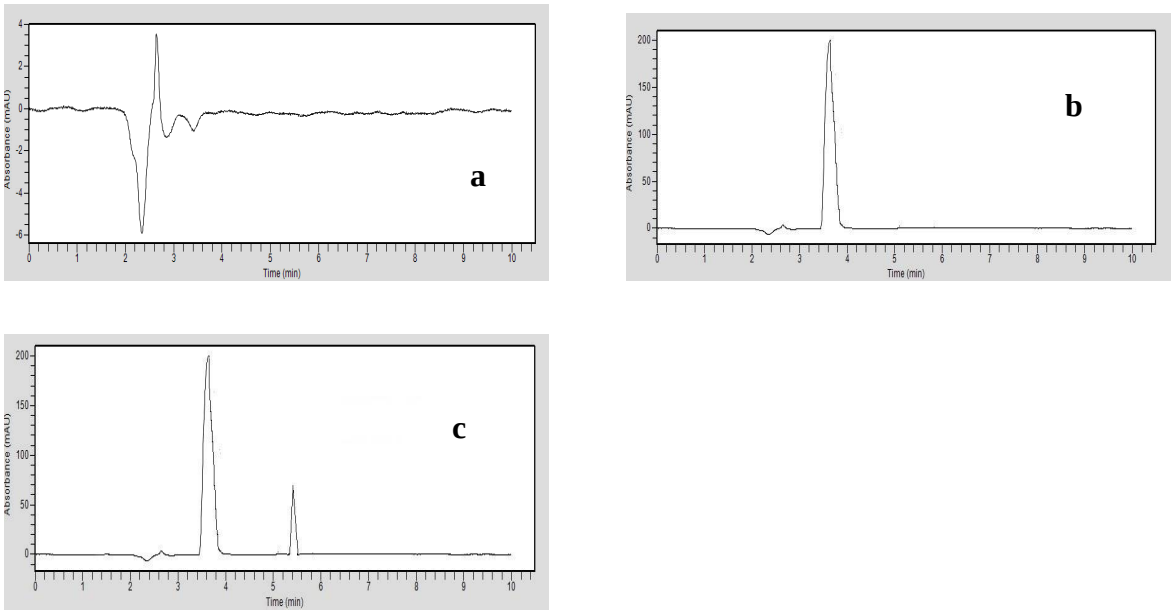

Figure 2. Chromatograms of (a) blank. (b) Osimertinib standard solution at 80 µg/ml. (c) Sample solution at 80 µg/ml.

Linearity: Linearity study of the method was performed at a concentration range of 64–96 µg/ml. The coefficient of determination (R^2) calculated for the plotted values was found over 0.999 which indicates linearity of the method (Figure 3).

Accuracy: Percent recovery method was put to use for determining the accuracy of the developed method. The study was executed by employing both the standard and sample solution. In all cases the recovery values were found within acceptable limit (Table 2).

Precision: Precision study was conducted by completing the experiment in intra-day and inter-day. The intraday and inter-day precision were 99.745±0.48 % and 99.51 ± 0.10 %, respectively which conformed with the acceptance limit (Table 3).

Sensitivity: After several trials of diluted solution of standard osimertinib, LOD (limit of detection) and

LOQ (limit of quantification) values found were 0.4 µg/ml and 1.2 µg/ml, respectively (figure 4a & 4b).

Ruggedness: The ruggedness assessment of the technique ensued through the execution of analyses by disparate analysts. The % RSD in the examination of robustness ranged from 100.013±0.06% to 100.018±0.034 (Table 4).

Robustness: Table 4 shows the findings of the robustness study regarding deliberate deviations in flow rate and mobile phase composition, where % recoveries range from 100.13 ± 0.06 to 100.18 ± 0.02 and 100.00 ± 0.021 to 100.13 ± 0.3, respectively.

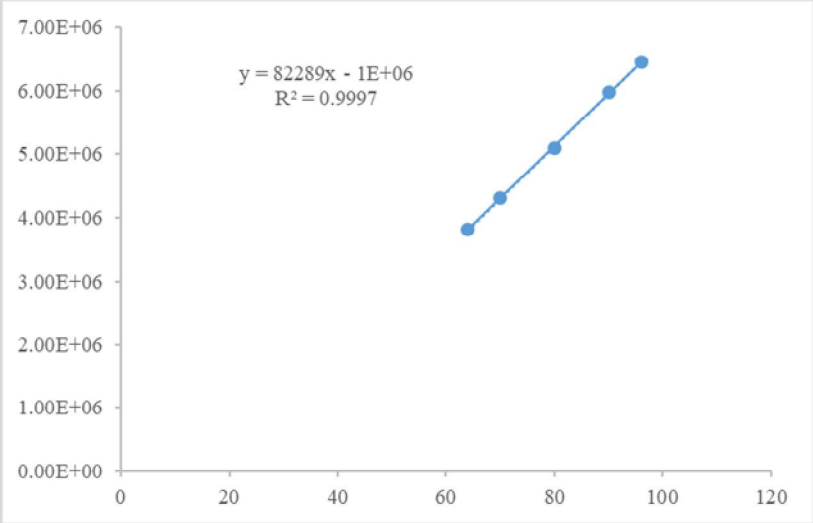

Figure 3. Linearity study for osimertinib at concentration range of 64-96 µg/ml.

Table 2. Result of accuracy study (n=3).

Type of Solution	Amount Added (µg/ml)	% Recovery (Mean ± % RSD)
Standard solution	70	99.92 ± 0.18
	80	100.23 ± 0.64
	90	100.13 ± 0.30
	Amount Added (µg/ml)	% Recovery (Mean ± % RSD)
Sample solution	70	100.12 ± 0.11
	80	99.32 ± 0.42
	90	100.37 ± 0.42

Table 3. Results of precision study.

Type of Solution	Amount Added (µg/ml)	Intra-day % recovery (Mean ± % RSD)	Inter-day (% Recovery ± % RSD)			Inter-day % recovery (Mean ± % RSD)
			Day 1	Day 2	Day 3	
Standard solution	80	100.013 ± 0.073	100.019 ± 0.073	99.937 ± 0.054	99.874 ± 0.065	99.943 ± 0.442
Sample solution	80	100.03 ± 0.40	99.91 ± 0.45	99.75 ± 0.10	99.87 ± 0.09	99.78 ± 0.61

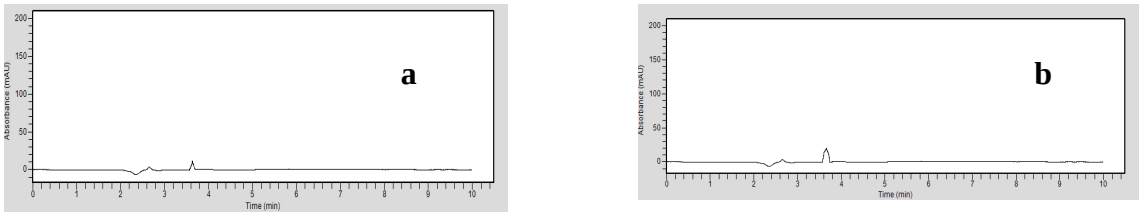

Figure 4. (a) Chromatograms found for the LOD determination (0.4 µg/ml). (b) LOQ determination (1.2 µg/ml).

Table 4. Results of robustness and ruggedness study.

Validation parameter	Parameters	Variations	Amount added (µg/ml)	Retention time (min)	% Recovery (Mean ± % RSD)
Ruggedness Study (n=6)	Analyst variation	Analyst 1	80	3.645	100.013 ± 0.06
		Analyst 2	80	3.637	100.018±0.034
Robustness Study(n=3)	Mobile phase flow rate (ml/min)	0.6	80	3.654	100.18 ± 0.02
		0.7	80	3.643	100.15 ± 0.06
		0.8	80	3.635	100.13 ± 0.06
	Mobile phase composition (%Buffer: % ACN)	63:37	80	3.649	100.13 ± 0.30
		65:35	80	3.641	100.013 ± 0.02
		67:33	80	3.632	100.00 ± 0.021

Conclusion

In this study, the established approach has incontrovertibly demonstrated to be validated with its simplicity and versatility for the ascertainment of osimertinib in bulk and formulation. Furthermore, the method uses easily accessible, inexpensive chemicals in a simple and non-cumbersome mobile phase composition. Hence, it can be used for the routine analysis and quality control of osimertinib and its dosage forms. Further research can also be done on osimertinib using this validated method.

Acknowledgement

The authors are grateful to Higher Education Quality Enhancement Project (HEQEP), AIF, Round-III, Window-2, CP-3245, Award No. 26, University Grants Commission (UGC), Bangladesh and Incepta Pharmaceuticals Ltd., Bangladesh for supporting the research work.

Conflict of interest

The authors are unanimous with conjoint interests.

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