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Research Article

A New Validated Stability Indicating UPLC Method for the Quantitative Analysis of Osimertinib Tablets

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Abstract

Objective: Osimertinib is an orally used kinase inhibitor for the treatment of cancer. A new stability indicating RP - UPLC method was developed for the quantification of Osimertinib tablets on gradient mode.

Materials and Methods: Acquit H-Class UPLC system (Waters, Milford, MA, USA) with a conditioned auto sampler and Acquity BEH C_{18} column (100 mm X 2.1 mm i.d., 1.7 µm particle size) (Waters, Milford, MA, USA) (column temperature 45°C) was operated with EMPOWER software for the present study. Mobile phase consisting of 0.1% formic acid: acetonitrile (mixed with 0.1% formic acid) was pumped at a flow rate of 0.40 mL/min and a detection wavelength of 271 nm with a 0.5 µL of injection volume. Osimertinib was subjected to stress degradation studies and the method was validated.

Results and Discussion: Osimertinib obeys Beer-Lambert's law 10 - 160 µg/ml with linear regression equation, y = 2189.2x + 1042.6 (R^2 = 0.9999). Osimertinib was found to be extremely sensitive towards basic hydrolysis and somewhat more sensitive towards oxidation.

Conclusion: The validated stability indicating method is very much sensitive and specific as Osimertinib drug peak was not at all

Keywords: Osimertinib; RP-UPLC; Stability Indicating; Validation; ICH Guidelines

Introduction

Osimertinib (Figure 1) is an anti-cancer drug acting as kinase inhibitor i.e. it inhibits the epidermal growth factor. It is available as tablets with label claim 40 mg and 80 mg. Osimertinib (C28H33N7O2) is chemically N-(2-{2 dimethyl amino ethyl-methyl amino}-4-methoxy-5-{[4-(1-methylindol-3-yl) pyrimidin-2yl] amino} phenyl) prop-2-enamide. It is a white crystalline powder with molecular weight 446.902 g/mol and pKa 5.4 and 7.2. Till now not even a single UPLC method has been reported in literature for the assay of Osimertinib. Osimertinib was determined by LC-MS [1] in human plasma, LC-MS/MS in rats [2] and HPLC [3] and in the present study a new stability indicating UPLC method has been developed for the quantitative analysis of Osimertinib within a period of 15 minutes and method was validated.

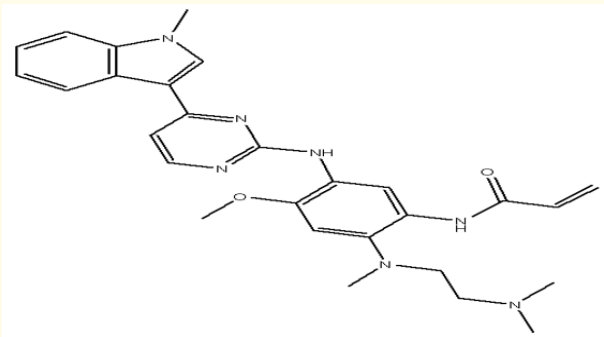

Figure 1: Chemical structure of Osimertinib.

Materials and Methods

Osimertinib pure drug (> 99.5 purity) and its process related substances were procured from MSN Laboratories Pvt Ltd. HPLC grade acetonitrile, formic acid, sodium hydroxide hydrochloric acid, and hydrogen peroxide were obtained from Merck. Ultrapure water was obtained from Milli-Q Gradient Millipore system. Stock solution of Osimertinib (1000 µg/ml) was prepared in acetonitrile and stored in refrigerator.

Chromatographic conditions

Chromatographic and mass spectral studies were performed by Acquity H-Class UPLC system (Waters, Milford, MA, USA) with a conditioned auto sampler, using an Acquity BEH C_{18} column (100 mm X 2.1 mm i.d., 1.7 µm particle size) (Waters, Milford, MA, USA). The column temperature was maintained at 45°C with EMPOWER software for the separation of related substances and conducting stress degradation studies. The mobile phase consisting of (A: B) 0.1% formic acid: acetonitrile (mixed with 0.1% formic acid) was pumped at a flow rate of 0.40 mL/min and a detection wavelength of 271 nm with a 0.5 µL of injection volume. The gradient elution program was as follows: 0 minute, 5% B; 1.80 minutes, 5% B; 3.20 minutes, 20% B; 6.10 minutes, 40% B; 8.00 minutes, 55% B; 10.30 minutes, 95% B; 13.00 minutes 95% B; 13.20 minutes 5% B.

Validation of analytical method [4]

Osimertinib solutions (10 - 160 µg/mL) were prepared and injected in to the UPLC system. The peak area of the chromatograms and thereby the mean peak area was noted, and calibration curve was drawn (mean peak area vs concentration of analyte). System precision (n = 10), intraday and inter day precision studies (n = 6) were performed for Osimertinib. Accuracy study was performed at 50%, 100% and 150% to that of LOQ by the addition of API to a fixed concentration of extracted tablet solution and % RSD was determined.

Assay of Osimertinib tablets

Osimertinib is available with brand names TAGRISSO (Label claim: 40 mg, 80 mg) (Astra Zeneca Pharma Ltd) and OSICENT (Label claim: 80 mg) (Unicorn Remedies). Tablets of each brand were procured, powdered and powder equivalent to 25 mg Osimertinib was weighed accurately and extracted with acetonitrile, filtered through membrane filter and later diluted with mobile phase as per the requirement and kept in refrigerator.

Stress degradation studies [5]

Osimertinib was subjected to three different stress conditions and the degradation products formed were separated using chromatographic analysis. Mass spectral studies were performed for quantification of the degradation products. Acidic hydrolysis (5N HCl at 80°C for 1hour), alkaline hydrolysis (0.001N NaOH) and oxidation (3% H_2O_2 at 80°C for 1 hour) reactions were performed with Osimertinib and those solutions were injected in to the system after neutralization.

Results and Discussion

A sensitive stability indicating UPLC method has been developed for the determination of Osimertinib (gradient mode) for the analysis of Osimertinib tablets. A brief scanned review of previous literature was given in table 1.

Mobile phase (v/v)	λ (nm)	Linearity (µg/ml)	Comments	Ref
Acetonitrile: water (1% formic acid) (Gradient mode)	-	-	LC/MS/ MS (human plasma)	1
Acetonitrile: water (0.1% formic acid) (Gradient mode)	-	-	LC/MS/ MS (Rat plasma)	2
Methanol: Phosphate buffer (50:50) (pH 3)	210	20.11 - 60.3	HPLC	3
Acetonitrile (containing 0.1% formic acid): 0.1% aq. formic acid (Gradient mode)	271	10 - 160	UPLC (Tablets)	Present method

Table 1: Comparison of present UPLC method with the reported methods.

Method optimization

Trails were made with changes in mobile phase composition, columns, flow rate, pH on isocratic mode but Osimertinib could not be separated and therefore gradient mode was chosen where a successful separation of both drug and its degradant products during the stability studies were observed. Two solvents were used where one of the solvents is a mixture of acetonitrile and 0.1% formic acid whereas the other solvent is only 0.1% formic acid. Stability studies were performed with flow rate 0.4 ml/min (PDA detection at 271 nm). Osimertinib was eluted at 5.960 minutes (Figure 2).

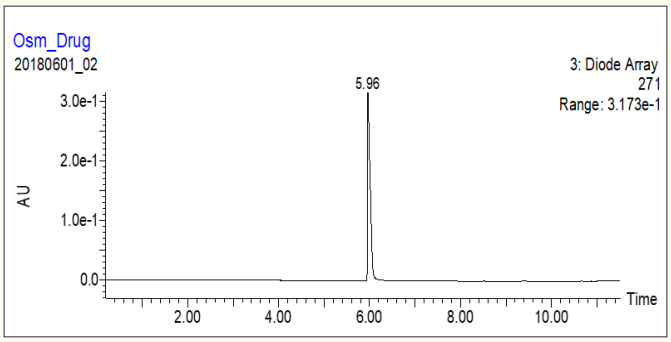

Figure 2: Specific chromatogram of Osimertinib (Rt 5.96 min) (1000 µg/mL).

Method validation and Assay

Linearity was observed over the concentration 10 - 160 µg/mL (Table 2). Osimertinib obeyed Beer-Lambert's law 10 - 160 µg/ml with linear regression equation, y = 2189.2x + 1042.6 (R^2 = 0.9999) (Figure 3). The % RSD in intraday and inter day precision and accuracy (Table 3) studies was less than 2 indicating that the method is precise and accurate. Osimertinib tablets has shown 99.83 - 99.97 % recovery. The LOD and LOQ were found to be 0.2971 and 0.9057 µg/ml.

Conc. (µg/ml)	*Mean peak area	% RSD
0	0	-
10	22131	0.1213
26	57972	0.1298
50	109673	0.2154
75	163962	0.2652
100	220347	0.2847
160	351638	0.2635

Table 2: Linearity study of osimertinib.

*Mean of six determinations.

*Level	*(% Recovery ± RSD)
LOQ	109.0 ± 0.58
50%	105.5 ± 0.45
100%	104.8 ± 0.57
150%	104.4 ± 0.44

Table 3: Accuracy study of osimertinib.

*Mean of three determinations.

Figure 3: Calibration curve of Osimertinib.

System suitability and solution stability of Osimertinib (25°C)

System suitability was determined by injecting Osimertinib 1000 µg/mL and the results were shown in table 4. Stability of Osimertinib standard solution was determined by injecting the standard Osimertinib solutions at different time intervals (1, 15, 20, 24 and 48 hrs) and found that the solutions are very much stable (Table 5).

Drug	Rt (min) ± SD	% RSD	RRT	Tailing factor	Theoretical plates
OSM	5.96 ± 0.018	0.31	1.000	1.216	66531

Table 4: System suitability of osimertinib.

RRT: Relative Retention Time.

Time (hrs)	Peak area	% Area variation
Initial	220347	Not applicable
21	217562	1.26
35	218612	0.79
44	218389	0.89

Table 5: Solution stability of Osimertinib (25°C).

Stress degradation studies and Specificity

Osimertinib has undergone degradations during acidic, alkaline and oxidation treatments. The stress degradation study has given a wide scope for the study of drug metabolism as the drug is available as tablet form. During oral administration the drug has to pass through different compartments of various pH due to peristaltic movement and therefore the present study directly or indirectly

helps one to evaluate the drug fate and its metabolites. The stress degradation reports and the system suitability parameters were also given were given in table 6.

Stress condition	% Degradation	Tailing Factor	Theoretical plates
Osimertinib (Untreated)	-	1.103	5382
Acidic degradation (5N HCl/80°C/1 hr)	7.16	1.201	5210
Alkaline degradation (0.001N NaOH)	99.08	1.362	4967
Oxidative degradation (3% H_2O_2/80°C/1hr)	50.07	1.109	5091

Table 6: Stress degradation studies of Osimertinib.

The UPLC chromatogram of Osimertinib during acidic degradation has shown the drug peak at Rt 5.950 min along with degradation products at 0.54, 5.420, 6.360 minutes (Figure 4) without interfering with the drug peak indicating that the method is specific. Osimertinib has shown less than 10% degradation (7.18%) during acidic degradation presenting that the drug is more stable towards acidic surroundings.

Figure 4: Specific UPLC chromatogram of Osimertinib during acidic degradation.

Osimertinib was reported to be extremely unstable in alkaline conditions (Figure 5). The degradation study was performed with NaOH initially 5N, 2N, 1N, 0.5N, 0.1N, 0.05N strengths but the drug was totally destroyed and therefore a very least concentration i.e. 0.001N NaOH was used to test the stability but almost 99.08 % of the Osimertinib was wiped out. But a small peak i.e. a degradation product was observed at Rt 5.490 minutes.

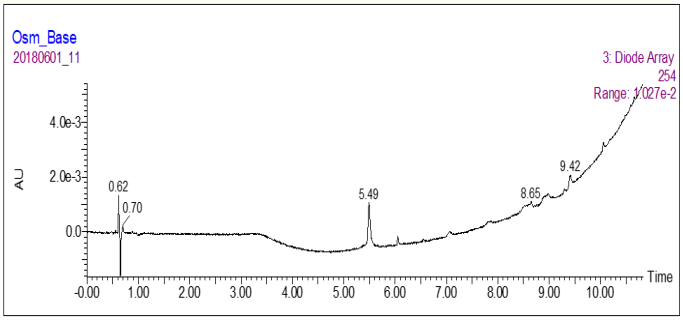

Figure 5: Specific chromatogram of Osimertinib during alkaline degradation.

The oxidation study was performed with H_2O_2 initially 30%, 25%, 20%, 15%, 10%, 5% strengths but the drug was totally destroyed and therefore at last 3% H_2O_2 was used where almost (Figure 6) about 50.07% of Osimertinib was destroyed but the drug peak (R_t 6.04 minutes) was not interfered with the degradant peaks aroused at 4.320, 4.620, 5.140, 5.230, 5.560, 5.990, 6.330, 6.540, 6.940 minutes. These reports indicating that Osimertinib is highly unstable, but the method is specific.

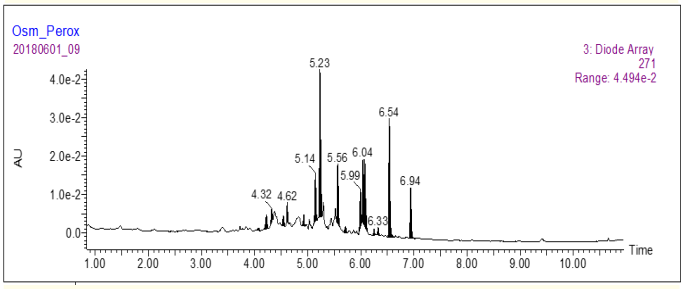

Figure 6: Specific UPLC chromatogram of Osimertinib during oxidative degradation.

Conclusion

The proposed UPLC study was very sensitive and extremely economical and can be very easily performed for the determination of Osimertinib tablets. The authors have done a quantitative determination as well as the degradation study for Osimertinib and found that the drug is highly sensitive towards alkaline and oxidation surroundings and finally the method was validated as per ICH guidelines. The present piece of work explores a wide scope for the study of drug metabolism of Osimertinib in cancer patients who are administered this drug orally and also kinetic studies (*in vivo and in vitro*).

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