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Original Article

RELIABLE SPECTROSCOPIC ANALYTICAL PROCEDURE FOR QUANTITATIVE ESTIMATION OF OSIMERTINIB MESYLATE IN LIPOSOMAL DRY POWDER INHALER FORMULATION

MANSING PATIL*, PINKAL PATEL

Department of Quality Assurance, Parul Institute of Pharmacy and Research, Parul University, P. O. Limda, Tal. Waghodia, Dist. Vadodara, Gujarat 391760, India

***Email: mansingpatil007@gmail.com**

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ABSTRACT

Objective: The objective of the present study was to develop an economical UV spectrophotometric method with a simple, rapid, accurate, precise, sensitive, and reproducible for the quantitative estimation of Osimertinib Mesylate (OM) in bulk and newly prepared Liposomal Dry Powder Inhaler (LDPI) formulation which has not been reported earlier.

Methods: Different dilutions were prepared in methanol in the range of 4-16 µg/ml, scanned between 400-200 nm, and determined the maximum absorbance was to confirm the drug's λmax. Linearity, accuracy, precision, the limit of detection (LOD), and the limit of quantitation (LOQ) were used as parameters to validate the method. The concentration of the OM was calculated based on a linear regression equation of the calibration curve.

Results: The UV spectrum of OM showed λmax at 267 nm and a linear calibration curve with a regression coefficient (R2) of more than 0.997. The RSD for recovery studies was found < 2 % and confirmed the accuracy of the proposed method. The LOD and LOQ were observed at 0.021 µg/ml and 0.063 µg/ml, respectively for bulk and 0.056 µg/ml and 0.170 µg/ml for OM LDPI formulation. The method was found to be precise with an RSD < 2 %.

Conclusion: The present UV spectrophotometric method can be used to successfully estimate OM in LDPI, and there is no interference of excipients during the study. The method is validated in compliance with International Conference on Harmonization (ICH) guidelines and it should be used as a routine quality control analysis i.e., assay for such dosage forms.

Keywords: Osimertinib mesylate, Drug content, Liposomes, Liposomal dry powder inhaler, UV Spectrophotometry, Method development, Validation, ICH

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INTRODUCTION

Osimertinib Mesylate (OM) is the third-generation of kinase inhibitor and is used for the first-line treatment of patients with metastatic Non-Small Cell Lung Carcinoma (NSCLC) whose tumors have epidermal growth factor receptor (EGFR) exon 19 deletions or exon 21 L858R mutations and the treatment of patients with metastatic EGFR T790M mutation-positive NSCLC, whose disease has progressed on or after EGFR Tyrosine Kinase Inhibitor (TKI) therapy [1, 2]. The mechanism action of OM binds irreversibly to certain mutant forms of EGFR (T790M, L858R, and exon 19 deletions) at approximately 9-fold lower concentrations than wild-type [3]. OM is a BCS class 3 (High Solubility Low Permeability) and has pKa are 9.5 (aliphatic amine) and 4.4 (aniline) [4, 5]. OM has pH-dependent aqueous solubility and is highly soluble in organic solvents i.e., methanol and DMSO [6]. Osimertinib liposomal vesicle formulation shows higher antitumor efficacy against cancer cells as compared to free osimertinib [7]. Liposomes and liposomal dry powder inhaler (LDPI) is the targeted drug delivery system to improve therapeutic efficacy and reduce unwanted side effects of the drug with dose frequency [8-11]. Inhalable osimertinib liposomes have increased potential therapeutic outcomes with limited systemic toxicity and an HPLC analytical method have adopted to estimate the osimertinib in the formulation [12]. Osimertinib mesylate liposomal dry powder inhaler is the proposed formulation and can be used for the treatment of NSCLC by inhalation administration effectively to improve therapeutic as well as patient compliance and convenience. However, all of these present methods are not employed for the determination of OM in a possible pharmaceutical dosage form i.e., liposomal dry powder inhaler, and are usually employed to quantify illicit substances in the formulation. A very big challenge in the development of a novel drug delivery system is the analytical procedure to estimate a drug in final formulation with no interferences of excipients. Simple economical method development and its validation is the primary objective of this study using UV spectrophotometry to estimate the assay or drug content of OM from possible pharmaceutical dosage forms derived from a newly prepared LDPI formulation which has not been reported earlier. The rationale of the study is to develop a UV spectrophotometric method with simple, rapid, accurate, precise, sensitive, and reproducible using OM. To achieve this purpose, the proposed analytical method has been used for the determination of the assay or drug content of OM in LDPI formulation.

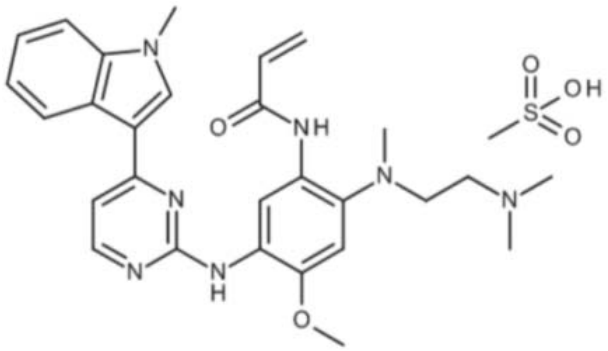

Fig. 1: Structure of osimertinib mesylate [13]

MATERIALS AND METHODS

Chemicals and instrumentation

Osimertinib Mesylate was obtained as a gift sample from MC Tech. Co., Ltd. Beijing, China. HPLC grade Methanol (Merck) was used [14].

A double-beam UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan) was used [14]. UV-VIS spectrophotometer connected with a computer loaded with UV Probe spectra manager 2.35 software was used for all spectral measurements with automatic wavelength corrections using a pair of 10 mm quartz cells. The spectra were obtained with the instrumental parameters as follows: Wavelength range: 400-200 nm. Sonicator (Sonica ultrasonic cleaner). All weights were taken on the electronic balance (Shimadzu).

Preparation of standard stock solution

The standard stock solution of OM was prepared by accurately weighing 10 mg of pure drug and was transferred into a 100 ml volumetric flask. A small amount of Methanol was added and sonicated for a few minutes to dissolve the remaining drug completely. Later, the drug solution was made up to 100 ml by adding the remaining amount of Methanol to give a concentration of 1000 µg/ml., which was used for further dilutions.

Procedure for the calibration curve

Several dilutions were prepared in the range of 4-16 µg/ml from the standard stock solution, where the beer's law was obeyed. The dilutions are 4 µg/ml, 6 µg/ml, 8 µg/ml, 10 µg/ml, 12 µg/ml, 14 µg/ml, 16 µg/ml respectively. Prepared different concentrations were scanned between 400 to 200 nm. The maximum absorbance was determined to confirm the λmax of the drugs using a UV-Vis Spectrophotometer.

Formulation sample preparation

Accurately weigh the appropriate amount of OM LDPI formulation equivalent to 2 mg of Osimertinib mesylate and transfer it into 100 ml of a volumetric flask. Add about 50 ml of Methanol and sonicate for a few minutes to dissolve it completely and make the volume up to the mark with Methanol. Further pipette 4 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with Methanol and the final concentration was prepared at 8 µg/ml. The samples were filtered through a 0.45 µm nylon syringe filter before analysis using a corrective and validated UV method. The absorbance of the sample solution was measured against a blank LDPI formulation (Formulation without drug) in methanol and readings were taken in triplicate. The concentration of the OM was calculated based on a linear regression equation of the calibration curve.

Method validation

Validation is the process of the analytical method by which it is established, by development studies, that the analytical performance characteristics of the method meet the requirements of intended analytical applications [15]. Typical analytical parameters verified in the analytical method validation are linearity, accuracy or recovery, precision, the limit of detection (LOD), and the limit of quantitation (LOQ). All the parameters were evaluated as per ICH guidelines [16].

Linearity

The calibration curves were developed with different concentrations of OM ranging from 4 µg/ml to 16 µg/ml. The calibration curves were developed by plotting the absorbance of OM against its concentrations. The linearity was evaluated by linear regression analysis, which was calculated by the lease square method.

Accuracy

The standard addition method is used for the determination of accuracy. Accuracy was performed as per the ICH guidelines by the percent recovery studies. The recovery studies were performed at three different concentrations of standard OM added to the sample solution with a known content of OM. The samples were prepared according to the "sample preparation", and the OM was spiked with 80 %, 100 %, and 120 % of the reference standard. The recovery studies were performed in triplicate for each concentration, and the accuracy of the method was expressed as the percent recovery and total mean recovery. The accuracy of an analytical method is the closeness of the test results obtained by that method to the true value and found value, which was evaluated as % variation for OM according to the following equation:

$$\text{Percentage Accuracy (\%)} = \frac{\text{Observed concentration}}{\text{Standard concentration}} \times 100$$

Precision

The precision of an analytical method is the closeness of agreement among individual test results when the method is repeatedly used for multiple samplings of the same sample. Generally expressed as the standard deviation or coefficient of variation of a series of measurements. Precision may be measured by the analytical method's degree of reproducibility or repeatability under normal operating conditions. Reproducibility represents the precision between different laboratories, as in a collaborative study. Repeatability represents the precision under the same operating condition within a laboratory over a short period. The precision of the analytical method was assessed concerning repeatability and reproducibility studies. The precision of the proposed analytical method was checked by Intra and inter-day repeatability study and expressed as % RSD among responses using the formula.

$$\%\ \text{RSD} = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ are the sensitivity of the proposed developed analytical method. LOD is the minimum amount of analyte in the sample that can be detected and LOQ is the minimum amount of analyte in the sample that can be measured with accuracy and precision. LOD and LOQ were calculated from the calibration curve. The LOD (k=3.3) and LOQ (k=10) of the proposed analytical method were calculated using the following equation [17]:

$$A = \frac{k\sigma}{S}$$

Where A is LOD or LOQ, σ is the standard deviation of the response, and S is the slope of the calibration curve.

RESULTS AND DISCUSSION

A new spectrophotometric method was developed for the estimation of OM in bulk and LDPI formulation. OM solution in methanol has observed maximum absorption (λmax) at 267 nm after scanning on a UV-Visible spectrophotometer which was reported as λmax in the literature for tablet dosage form [14]. The observed corresponding absorption spectra were shown in fig. 2. The correlation coefficient (r^2) was found to be acceptable. The drug Osimertinib mesylate showed linearity between 4 µg/ml to 16 µg/ml concentration. The method was validated by linearity, accuracy, precision, LOD, and LOQ. The % recovery and % RSD were found to be within acceptable limits for accuracy and precision, respectively, representing that the developed method was admissible. Also, LOD and LOQ were found within an acceptable level. The results indicate that the developed method is satisfactory for the drug OM.

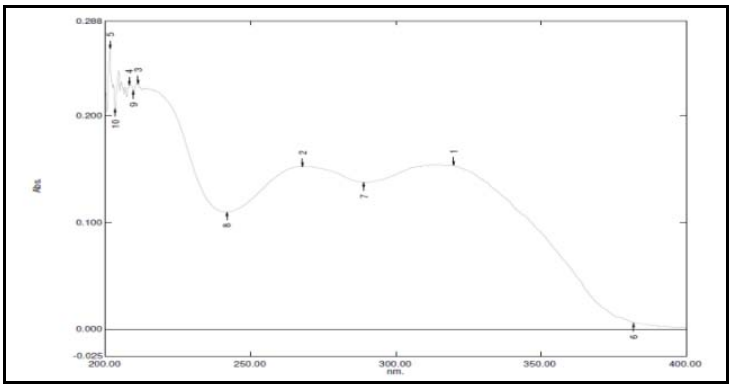

Fig. 2: Absorption spectrum of osimertinib mesylate in methanol

Method validation

The validation was performed on the developed method with the following parameters:

Linearity

The linearity of the bulk method was found to be linear over a wide concentration range of 4 µg/ml to 16 µg/ml concentration with a regression equation of "$y = 0.0572x + 0.0205$" and found an excellent correlation coefficient of 0.9973. The sample solution absorbance was measured against plain methanol, which was presented in table 1. The absorbance has been taken against the concentration for the construction of the calibration curve and is shown in fig. 3.

The linearity for OM LDPI formulation was found to be linear over a wide concentration range of 4 µg/ml to 16 µg/ml concentration with a regression equation of "$y = 0.0568x + 0.0071$" and found an excellent correlation coefficient of 0.9994. The sample solution absorbance was measured against a formulation without the drug in methanol and which was presented in table 1. The absorbance has been taken against the concentration for the construction of the calibration curve and is shown in fig. 4.

Table 1: Absorbance for OM and OM LDPI formulation in methanol

Concentration (µg/ml)	Absorbance*	
	Osimertinib mesylate	Osimertinib mesylate liposomal dry powder inhaler
4	0.2435±0.001	0.2331±0.003
6	0.3960±0.001	0.3644±0.001
8	0.4875±0.001	0.4585±0.002
10	0.5923±0.001	0.5727±0.003
12	0.7045±0.003	0.6891±0.001
14	0.8186±0.002	0.7963±0.002
16	0.9222±0.001	0.9161±0.001

*All values are presented as mean n=3±standard deviation (SD)

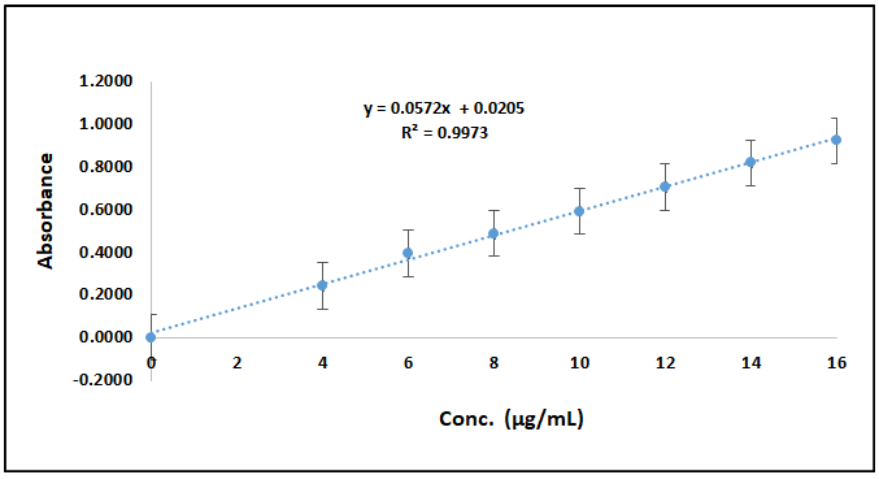

Fig. 3: Calibration curves of OM in methanol (n=3)

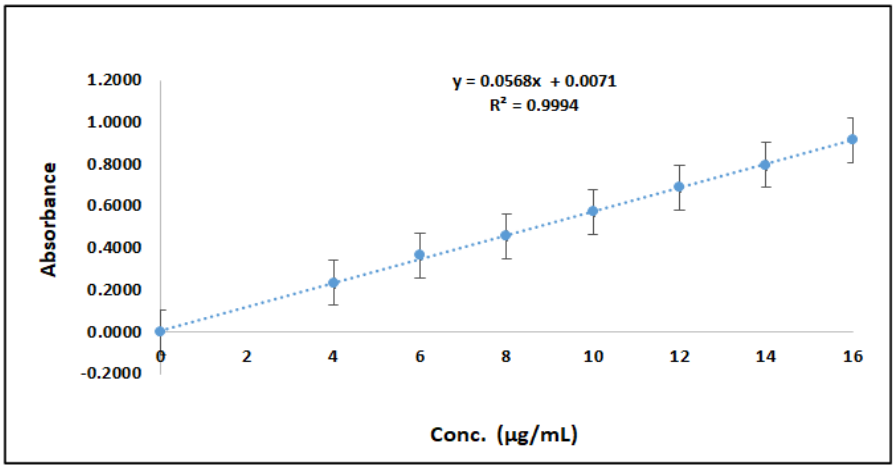

Fig. 4: Calibration curves of OM LDPI in methanol (n=3)

Accuracy

The percentage recovery for bulk was found to be within the acceptable range from 98.16 % to 101.46 % with a % RSD less than 2 for all 9 samples. The results were presented in table 2 and imply the high accuracy of the method.

The percentage recovery for OM LDPI formulation was found to be within the acceptable range from 98.81 % to 101.07 % with a % RSD less than 2 for all 9 samples. The results were presented in table 2 and imply the high accuracy of the method.

Precision

A precision study for bulk and OM LDPI formulation was performed for the developed method by evaluating intra-day and inter-day variations. In an intra-day precision study, prepared different concentrations of solutions and measured absorbance's thrice a day i.e., morning, afternoon, and evening. In an inter-day precision study, prepared different concentrations of solutions and analyzed on three consecutive days, i.e., day 1, day 2, and day 3. The results for Intra and inter-day precision study were presented in table 3, respectively and the % RSD was found less than 2, which indicates the drug or sample solution is stable for a day and 3 d implies high accuracy of the method.

Limit of detection (LOD) and limit of quantitation (LOQ)

The sensitivity of the proposed analytical method was evaluated in terms of the Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOD and LOQ of OM by the proposed method was found at 0.021 µg/ml and 0.063 µg/ml for bulk, respectively, and 0.056 µg/ml and 0.170 µg/ml for OM LDPI formulation, respectively. The LOQ and LOQ value shows that the method can be applied to determine the minimum concentration of drug in bulk as well as formulation.

Assay of OM loaded LDPI formulation

The validated UV spectrophotometric method was applied for the analysis of OM loaded LDPI formulation. The percentage assay values of OM in LDPI formulations were found to be ranged from 98.58 % to 101.07 % and % RSD not more than 2, and the results were represented in table 4. Interfering spectrum were not observed; hence its clearly indicating that there was no interference in the excipient used in LDPI formulation.

The values of an assay or drug content in formulations were the same as mentioned in the label claim. The results of the assay indicate that the method is selective for the assay of OM without interference from the lipids and excipients used in the dosage form with respective stability. The evaluated drug content in formulation with low relative standard deviation values established the precision of the proposed method.

Table 2: Accuracy or recovery study of OM and OM LDPI formulation in methanol

Concentration level (%)	Concentration (µg/ml)	Recovery of osimertinib mesylate		Recovery of osimertinib mesylate liposomal dry powder inhaler	
		% Recovery*	% RSD	% Recovery*	% RSD
80	10	98.92±0.33	0.33	99.61±1.27	1.28
100	12	98.63±0.43	0.44	98.50±1.54	1.57
120	14	100.26±1.23	1.23	99.12±0.51	0.52

*All values are presented as mean n=3±standard deviation (SD)

Table 3: A precision study of OM and OM LDPI formulation in methanol

Concentration (µg/ml)	Precision study for osimertinib mesylate				Precision study for osimertinib mesylate liposomal dry powder inhaler			
	Intra-day precision study		Inter-day precision study		Intra-day precision study		Inter-day precision study	
	Absorbance*	% RSD	Absorbance*	% RSD	Absorbance*	% RSD	Absorbance*	% RSD
10	0.6076±0.006	0.94	0.5982±0.008	1.37	0.5923±0.001	0.09	0.6006±0.011	1.75
12	0.7139±0.002	0.28	0.7064±0.007	0.96	0.7045±0.003	0.38	0.7072±0.012	1.66
14	0.8265±0.002	0.27	0.8209±0.005	0.60	0.8186±0.002	0.21	0.8186±0.013	1.55

*All values are presented as mean n=3±standard deviation (SD)

Table 4: % Assay of OM LDPI formulation

Stability study		% Assay*	% RSD
Storage condition	**Duration**		
Initial	Initial	101.07±0.12	0.13
Accelerated (40 °C±2 °C/75 % RH±5 %)	3 Mo	98.58±1.35	1.35
Intermediate (30 °C±2 °C/65 % RH±5 %)	3 Mo	99.47±0.18	0.18
Controlled room temperature (25 °C±2 °C/60 % RH±5 %)	3 Mo	98.83±0.29	0.29

*All values are presented as mean n=3±standard deviation (SD)

The previously reported methods for estimation of OM only for tablet dosage form using UV spectrophotometric and HPLC method [14, 18]. A new UV spectrophotometric method was developed and validated for the quantification of OM in LDPI formulation. The newness of this method is an estimation of drug OM in LDPI formulation, where LDPI formulation of OM has not been prepared previously as well as OM was not estimated in LDPI formulation as per previous study reports. The results of various validation parameters showed that the newly developed UV spectrophotometric method is suitable for the quantitative determination of OM in LDPI formulation. Hence the validated UV spectrophotometric method can be readily adapted for the estimation of OM in LDPI formulation. The new analytical method was linear in an almost wide concentration range (4 µg/ml to 16 µg/ml) with an accepted standard deviation as compared to the previously reported method for the estimation of OM in tablet dosage form using UV spectrophotometric [14]. Accuracy is a very important validation factor, which was reported as percent recovery. The accuracy of the previous HPLC method for the estimation of OM in tablet dosage form was reported between 99.00-100.20%; however, the present method for the estimation of OM in LDPI formulation was found in the range of 98.50% to 99.61 % recovery and with less than 2 % RSD [18]. The precision of the method was found satisfactory as compared to previously reported methods reported for the estimation of OM in LDPI formulation using UV spectrophotometric method [14, 18]. The LOD and LOQ of the previous UV spectrophotometric method for the estimation of OM in tablet dosage form was reported 0.3 µg/ml and 0.99 µg/ml, respectively; however, the present method for the estimation of OM in LDPI formulation was found 0.021 µg/ml and 0.063 µg/ml respectively and based on the results we can conclude that the present method is very sensitive as compared to previously reported method [14]. The percentage assay for OM LDPI formulation was found to be 98.52 % to 101.07 % with respective initial as well as stability. Further, no interfering spectrum were observed for OM LDPI formulation, clearly indicating that there was no interference in the excipient used in LDPI formulation. Since no UV spectrophotometric and HPLC method is available for the estimation of OM in LDPI formulations, we believe that the obtained results with UV spectrophotometric method are adequate for the previously defined objectives. The obtained results suggest that this analytical procedure could be successfully applied for the estimation of Osimertinib mesylate in LDPI formulation without excipients interferences. The method is very economical as compare to HPLC and UPLC method and require common solvent with simple laboratory equipment. The developed method is convenient and effective for quality control as well as routine analysis of OM in the LDPI formulation.

CONCLUSION

The present UV spectrophotometric method can be used for the estimation of Osimertinib mesylate in liposomal dry powder inhalers successfully and there is no interference of excipients during the study. The method is validated in compliance with ICH guidelines and should be used as a routine quality control analysis for such dosage forms as simple, rapid, reliable, and economical.

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AUTHORS CONTRIBUTIONS

The listed authors have contributed equally to this manuscript.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest for the presented research work.

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