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UV METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF AZELNIDIPINE AND OLMISARTAN IN FIXED DOSE COMBINATION

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ABSTRACT

A simple, rapid, and reliable UV spectrophotometric method was developed and validated for the simultaneous estimation of Azelnidipine (ALD) and Olmesartan (OST) in fixed dose combination tablet dosage form. The method was based on the simultaneous equation approach using two selected wavelengths. The maximum absorption (λ_{max}) of Azelnidipine and Olmesartan were found to be 264 nm and 282 nm respectively. The linearity of the method was established in the concentration range of 5–25 µg/ml for both drugs with correlation coefficients of 0.9999 for ALD and 0.9993 for OST, indicating excellent linearity. The developed method was validated according to analytical validation guidelines and parameters such as accuracy, precision, repeatability, intermediate precision, and reproducibility were evaluated. The percentage recovery values ranged from 98.31% to 98.78% for ALD and 98.33% to 98.94% for OST, demonstrating the accuracy of the method. Precision studies showed %RSD values less than 2%, confirming the reliability and reproducibility of the method. The developed method was successfully applied for the analysis of marketed tablet formulations and the percentage assay results were found to be within acceptable limits. Therefore, the proposed UV spectrophotometric method is simple, precise, accurate, and suitable for routine quality control analysis of Azelnidipine and Olmesartan in combined pharmaceutical dosage forms.

Keywords: Azelnidipine, Olmesartan, UV Spectrophotometry, Simultaneous Estimation, Method Validation, Linearity, Accuracy, Precision, Fixed Dose Combination, Pharmaceutical Analysis.

INTRODUCTION

Hypertension is one of the most prevalent cardiovascular disorders worldwide and is a major risk factor for stroke, myocardial infarction, and renal failure. Effective management of hypertension often requires combination therapy involving drugs with different mechanisms of action to achieve better therapeutic outcomes (Burnier and Damianaki, 2023).

Fixed-dose combinations (FDCs) are widely used in antihypertensive therapy because they improve patient compliance, enhance therapeutic efficacy, and reduce adverse effects compared with monotherapy (Gupta *et al.*, 2010).

Azelnidipine is a long-acting dihydropyridine calcium channel blocker that exerts its antihypertensive effect by inhibiting calcium ion influx through L-type calcium channels in vascular smooth muscle, resulting in

vasodilation and reduction of peripheral vascular resistance. It has been reported to produce a gradual and sustained reduction in blood pressure with minimal reflex tachycardia (Ram, 2022; Fujisawa *et al.*, 2009).

Olmesartan Medoxomil is an angiotensin II receptor antagonist that selectively blocks the AT_1 receptor, thereby preventing vasoconstriction and aldosterone secretion induced by angiotensin II. This leads to a decrease in systemic vascular resistance and effective control of hypertension. The combination of azelnidipine and olmesartan provides a synergistic antihypertensive effect because the drugs act through complementary mechanisms (Mire *et al.,* 2025).

For quality control and routine analysis of pharmaceutical formulations, the development of simple, rapid, and reliable analytical methods is essential (Masson, 2007). Among various analytical techniques, UV-Visible spectrophotometry is widely used due to its simplicity, cost-effectiveness, accuracy, and suitability for routine laboratory analysis. Simultaneous estimation methods using UV spectroscopy allow the quantification of two drugs in combined dosage forms without the need for complex separation techniques (Hollein *et al.,* 2007).

Analytical method validation is a critical step in pharmaceutical analysis to ensure that the developed method is reliable and suitable for its intended purpose (Sharma *et al.,* 2018). According to the guidelines of the International Council for Harmonisation, validation parameters such as linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and robustness must be evaluated.

Therefore, the present study aims to develop and validate a simple, precise, and accurate UV spectrophotometric method for the simultaneous estimation of azelnidipine and olmesartan in fixed-dose combination formulations. The developed method can be effectively applied for routine quality control analysis in pharmaceutical industries and research laboratories.

MATERIALS AND METHODS

Materials

Azelnidipine (ALD) and Olmesartan (OST) pure drug samples were obtained as gift samples from a reputed pharmaceutical manufacturer. Methanol and distilled water of analytical reagent grade were used as solvents for the preparation of standard and sample solutions. Marketed tablet formulations containing Azelnidipine and Olmesartan were procured from the local pharmacy for analysis. Whatman filter paper No. 41 was used for filtration of the prepared solutions. All chemicals and reagents used in the investigation were of analytical grade and were used without further purification. The absorbance measurements were carried out using a UV–Visible spectrophotometer equipped with matched quartz cells.

Methods

Combination

Azelnidipine and Olmisartan combination launched in the market for the treatment of high blood pressure in the strength of 20:8mg. Till date there is no cost effective method for the spectrophotometric method for the estimation of Azelnidipine and Olmisartan in combination. Following are the marketed formulation to be estimated by using UV (Naulay *et al.*, 2015).

Linearity range and calibration graph

Preparation of Standard Stock Solution (Stock-A)

Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 80mL Methanol: water (80:20v/v) and the flask was sonicated for about 10 min to solubilize the drug and the volume was made up to the mark with Methanol: water (80:20v/v) to get a concentration of 1000 µg/ml (Stock-A) for both drugs.

Preparation of Sub Stock Solution (Stock-B)

Aliquots of 2.5 ml withdrawn with help of pipette from standard stock solution A of ALD and OST and transferred into 25 ml volumetric flask separately and diluted up to 25 ml with Methanol: water (80:20v/v) that gave concentration of 100 µg/ml (Stock-B).

Preparation of Working Standard Solution

Aliquots of 0.5 ml, 1.0 ml, 1.5 ml, 2 ml and 2.5 ml withdrawn with help of pipette from standard stock solution (Stock-B) separately in 10 ml volumetric flask and volume was made up to 10 ml with Methanol: water (80:20v/v). This gave the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml respectively for ALD (Grewal *et al.,* 2012).

0.5 ml, 1.0 ml, 1.5 ml, 2 ml and 2.5 ml from sub stock solution (Stock-B) were taken separately in 10 ml volumetric flask and volume was made up to 10 ml with Methanol: water (80:20v/v). This gave the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20 µg/ml and 25µg/ml respectively for OST.

Selection of wavelength for linearity

Solutions of 10µg/ml of ALD and 10 µg/ml OST were prepared separately. Both the solutions were scanned in the spectrum mode from 200 nm to 400 nm. The maximum absorbance of ALD and OST was observed at 276.0 nm and 230.0 nm, respectively. ALD and OST showed linearity in the concentration range of 10-50µg/ml and 5-25µg/ml at their respective maxima. Calibration curve was plotted, absorbance versus concentration.

Simultaneous equation method

Study of Overlay Spectra

Working standard solution from the standard stock solution prepared as in concentration 10 µg/ml of ALD and 10 µg/ml of OST were scanned in the spectrum mode over the range of 200-400 nm against Methanol: water (80:20v/v) as blank and the overlain spectra of the two were recorded. ALD showed an absorbance peak at 264.0 nm, whereas OST at 284.0 nm. The overlain spectra also showed isoabsorptive points at 273.0 nm (Krishna *et al.,* 2014).

Due to difference in absorbance maxima and having no interference with each other so both drug can be simultaneously estimated by simultaneous equation method (Gandhi *et al.,* 2013).

Simultaneous equation method is based on the absorption of drugs (X and Y) at the wavelength maximum of the other. Two wavelengths selected for the method are 264.0 nm and 282 nm that are λ_{max} of ALD and OST respectively. The absorbances were measured at the selected wavelengths and absorptivities ($A^{1\%,\ 1cm}$) for both the drugs at both wavelengths were determined as mean of five independent determinations. Concentrations in the sample were obtained by using following equations.

$$C_{ALD} = \frac{A_1ay_2 - A_2ay_2}{ax_1ay_2 - ax_2ay_1} \quad \text{..... Eq. (1)}$$

$$C_{OST} = \frac{A_1ax_2 - A_2ax_1}{ax_1ay_2 - ax_2ay_1} \quad \text{..... Eq. (2)}$$

Where, A_1 and A_2 are absorbances of mixture at 264.0 nm and 282.0 nm respectively, ax_1 and ax_2 are absorptivities of ALD at λ_1 (264.0 i.e. λ_{max} of ALD) and λ_2 (282.0 i.e. λ_{max} of OST) respectively and ay_1 and ay_2 are absorptivities of OST at λ_1 and λ_2 respectively. C_{ALD} and C_{OST} are concentrations of ALD and OST respectively. Figure 6.5 represent the overlain spectra of both the drugs in 8:20 ratio and the criteria for obtaining maximum precision [i.e. absorbance ratio $(A_2/A_1)/ax_2/ax_1$ and ay_2/ay_1] by this method were calculated and found to be outside the range of 0.1-2.0 which is satisfied for both the ALD and OST.

Validation of Simultaneous Equation Method (ICH; 2005)

Linearity

Linearity of both drugs was established by response ratios of drugs. Response ratio of drug calculated by dividing the absorbance with respective concentration. Then a graph was plotted between concentration and response ratio.

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of ALD and OST to preanalysed tablet solutions. The resulting solutions were then re-analysed by proposed methods. Whole analysis procedure was repeated to find out the recovery of the added drug sample. This recovery analysis was repeated at 3 replicate of 5 concentrations levels (Sharma *et al.*, 2023).

Precision

Precision of the methods was studied at three level as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility. Repeatability was performed by analyzing same concentration of drugs for five times. Day to Day was performed by analyzing 5 different concentration of the drug for three days in a week.

Analysis of tablet sample

Twenty marketed tablets of ALD and OST were weighed and ground to a fine powder; amount equal to 4mg of ALD was taken in 10 ml volumetric flask. The OST present in this amount of tablet powder was 10mg. Then 20ml of Methanol: water (80:20v/v) was added and the flask was sonicated for about 10 min to solubilize the drug present in tablet powder and the volume was made up to the mark with hydrotropic solution. After sonication filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with Methanol: water (80:20v/v) to get the final concentrations of both drugs in the working range. The absorbances of final dilutions were observed at selected wavelengths and the concentrations were obtained from Simultaneous Equation Method. The procedure was repeated for five times.

RESULTS AND DISCUSSION

The UV spectrophotometric method was successfully developed and validated for the simultaneous estimation of Azelnidipine (ALD) and Olmesartan (OST) in fixed dose combination tablets.

The selection of analytical wavelengths was carried out by scanning individual drug solutions in the UV region. ALD showed maximum absorbance (λmax) at 264 nm, while OST exhibited λ_{max} at 282 nm. The overlay spectra of both drugs demonstrated adequate spectral separation, allowing accurate simultaneous analysis without significant interference.

The linearity of the developed method was evaluated in the concentration range of 5–25 µg/ml for both drugs. The calibration curves showed excellent linear relationships between absorbance and concentration with regression equations $y = 0.0182x + 0.0025$ for ALD and $y = 0.0492x + 0.0011$ for OST. The correlation coefficients ($r^2 = 0.9999$ for ALD and 0.9993 for OST) confirmed the high degree of linearity of the method.

Accuracy of the method was determined by recovery studies at 80%, 100%, and 120% levels. The percentage recovery values ranged from 98.31% to 98.78% for ALD and 98.33% to 98.94% for OST, indicating that the method is highly accurate and free from interference of excipients present in the formulation. The low standard deviation values further confirmed the reliability of the method.

Precision was assessed in terms of repeatability, day-to-day precision, analyst-to-analyst precision, and reproducibility. The percentage relative standard deviation (%RSD) values for all precision parameters were found to be less than 2%, which indicates good precision and reproducibility of the analytical procedure.

The developed method was also applied for the analysis of marketed tablet formulations containing ALD and OST. The percentage assay values were found to be 99.61% for ALD and 98.90% for OST, which are within acceptable pharmacopeial limits. The low %RSD values further confirmed the suitability of the method for routine quality control analysis.

The results indicate that the developed UV spectrophotometric method is simple, accurate, precise, and reliable for the simultaneous estimation of Azelnidipine and Olmesartan in pharmaceutical dosage forms. The method can therefore be effectively used for routine analysis and quality control of fixed dose combination formulations.

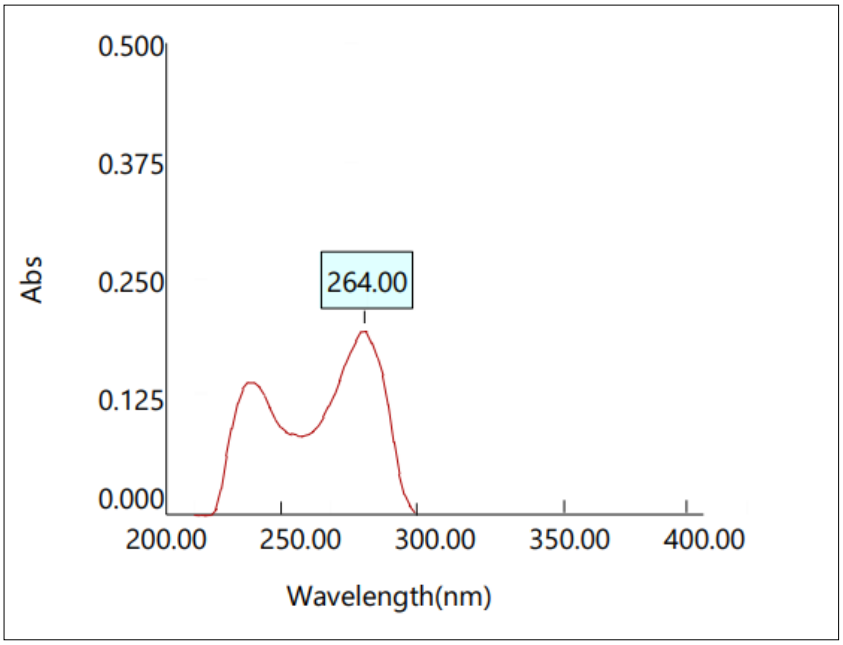

Figure 1: Selection of λ_{max} of ALD

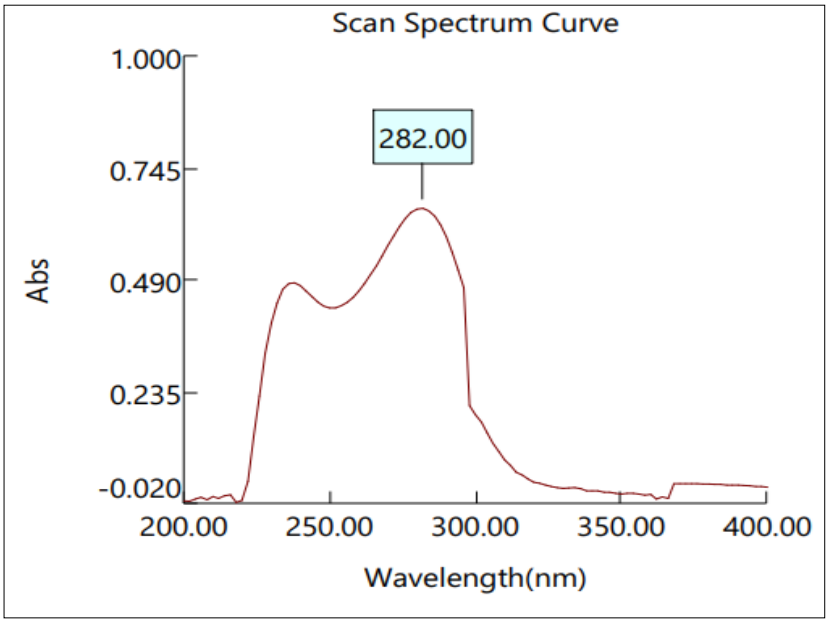

Figure 2: Selection of λ_{max} of OST

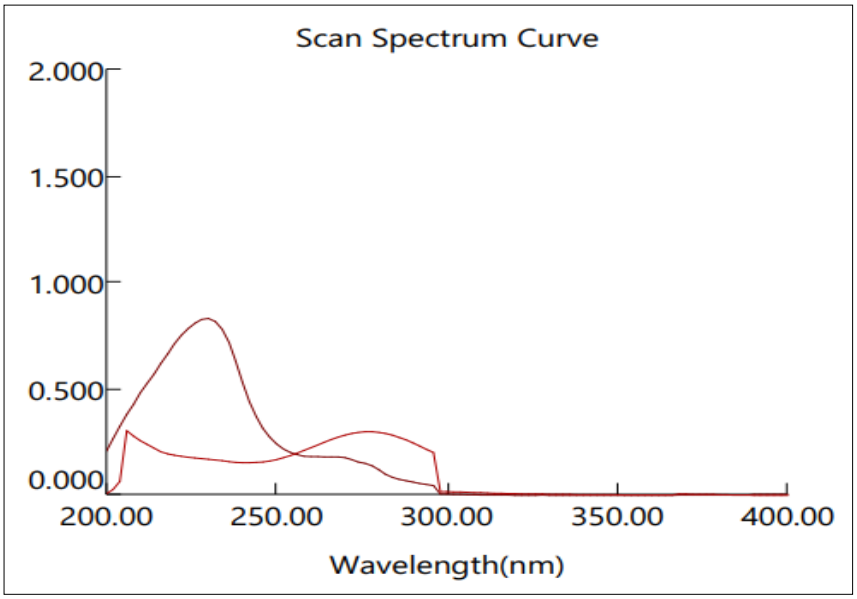

Figure 3: Overlay Spectra of ALD and OST

Table 1: Statistical Analysis of Linearity Data for ALD and OST

Parameter	ALD (λ_{max} = 264 nm)	OST (λ_{max} = 282 nm)
Linearity Range (µg/ml)	5–25	5–25
Regression Equation	y = 0.0182x + 0.0025	y = 0.0492x + 0.0011
Slope (m)	0.0182	0.0492
Intercept (c)	0.0025	0.0011
Correlation Coefficient (r^2)	0.9999	0.9993
Mean Absorbance Range	0.1468 – 0.7164	0.246 – 1.215

Table 2: Results of Method Validation Parameters for Simultaneous Estimation of ALD and OST

Validation Parameter	ALD (Azelnidipine)	OST (Olmesartan)
Accuracy (Recovery %)		
80% Level (Mean ± SD)	98.67 ± 0.666	98.94 ± 0.798
100% Level (Mean ± SD)	98.78 ± 0.653	98.70 ± 0.513
120% Level (Mean ± SD)	98.31 ± 0.907	98.33 ± 1.075
Precision (%RSD)		
Repeatability	0.079	0.143
Day-to-Day Precision	0.104	0.103
Analyst-to-Analyst Precision	0.106	0.093
Reproducibility	0.141	0.145
Assay of Marketed Formulation		
Mean % Assay	99.61	98.90
% RSD	2.065	1.069

CONCLUSION

A simple, accurate, precise, and cost-effective UV spectrophotometric method was successfully developed and validated for the simultaneous estimation of Azelnidipine (ALD) and Olmesartan (OST) in fixed dose combination tablets. The method showed excellent linearity within the concentration range of 5–25 µg/ml for both drugs with high correlation coefficients. Validation parameters such as accuracy, precision, repeatability, and reproducibility were found to be within acceptable limits, indicating the reliability of the developed method. The percentage recovery values were close to 100%, confirming the accuracy of the method. Therefore, the developed UV method can be effectively applied for routine quality control analysis of Azelnidipine and Olmesartan in pharmaceutical formulations.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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