



## SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS DETERMINATION OF OLMESARTAN MEDOXOMIL AND AMLODIPINE BESYLATE FROM TABLET DOSAGE FORM

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### ABSTRACT

Simple, sensitive and specific spectrophotometric method were developed and validated for quantitation of Olmesartan Medoxomil (OLM) and Amlodipine Besylate (AML) in tablet dosage form. The new analytical absorption correction method was developed based on the simultaneous estimation of drugs in a binary mixture without previous separation. The method is based upon determination of OLM at 265 nm and AML at 360 nm in ACN: Water. The system obeyed Beers law over the concentration of 2 to 32 µg ml<sup>-1</sup> for OLM and 2 to 20 µg ml<sup>-1</sup> for AML. Interday and intraday studies showed repeatability of the method. Results of tablet analysis in the range of 98.15 to 100.15% and 99.67 to 100.88% for OLM and AML, which indicate repeatability of the method. The recoveries from 101.99 to 100.25 % and 99.66 to 101.15 % for OLM and AML which do not differ from 100% showed that there was no interference from common excipients indicates accuracy and reliability of the method.

**Keywords:** Amlodipine besylate, Olmesartan medoxomil, Spectroscopy.

### INTRODUCTION

Market survey revealed that, day by day new drugs and their combination with another drugs are being introduced in market as they have more patient compliance than a single drug. The analytical chemistry hence has challenge in developing the methods for their analysis with the help of number of analytical techniques which are available for the estimation of the drugs and their combination. Analytical Chemistry involves separations, identification and determination of the relative amount of the component in a sample matter. In Analytical Chemistry 'Quality' is associated with accuracy and reproducibility; other criteria can be cost, speed and information. Analytical monitoring of pharmaceutical product or of specific ingredients within the product is necessary to ensure the safety and efficacy throughout the shelf life, including storage, distribution and use.<sup>1</sup> In general terms pharmaceutical analysis comprises, those procedures necessary to determine the identity, strength, quality and purity of drugs and chemicals.

Olmesartan Medoxomil (OLM) and Amlodipine Besylate (AML) are recently introduced in the market as combined tablet dosage form which is widely used in the treatment of hypertension.<sup>2-3</sup> Olmesartan Medoxomil 4-(1-Hydroxy-1-methylethyl)-2-propyl-1-[[2'-(1H-tetrazol-5-yl) [1, 1'-biphenyl] - 4-yl] methyl-1H-imidazole-5-carboxylic acid (5-methyl-2-oxo -1,3-dioxol-4-yl) methyl ester. Olmesartan works by blocking the binding of angiotensin II to the AT<sub>1</sub> receptors in vascular muscle. As a result of the blockage, Olmesartan restrict vasoconstriction and the secretion of aldosterone. This reduces blood pressure by causing vasodilation and reducing peripheral resistance. Amlodipine Besylate is chemically 2-[[2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4 dihydro-6-methyl-3,5- pyridine dicarboxylic acid -3 ethyl-5- methyl ester monobenzene sulphonate and it is a peripheral arterial vasodilator that acts directly on vascular smooth muscle to cause a reduction in peripheral vascular resistance and reduction in blood pressure.

There is various methods as UV<sup>4</sup>, HPTLC<sup>5</sup>, Mass<sup>6</sup>, LC-MS-MS<sup>7</sup>, CZE<sup>8</sup>, HPLC<sup>9,10</sup> for OLM alone or in combination with other dosage form and also for AML alone or in combination with other dosage form such as HPLC<sup>11-26</sup>, HPTLC<sup>27</sup>, UV<sup>28-32</sup> for simultaneous estimation. But there is no method reported for simultaneous estimation of OLM and AML from dosage forms by RP-HPLC. In the analysis of formulations containing two or more drugs, one drug can interfere in the estimation of another drug. To avoid this, separation of components of mixture by extraction is usually carried out which make the procedure time consuming and complicated and often lacks accuracy.

The present work was undertaken to develop such method of analysis, which can estimate both the drugs in combination without

prior separation which is a precise, accurate, simple, reliable and less time consuming method for estimation of drugs in tablet.

### MATERIALS AND METHODS

#### Apparatus

The instrument used for the present study was PC based Jasco V-530 UV-Visible double beam Spectrophotometer with 1 cm matched pair quartz cell and spectral bandwidth of 2 nm.

#### Reagents and materials

Olmesartan Medoxomil and Amlodipine Besylate were obtained as a gift sample from Cipla, Vapi, India. Acetonitrile were purchased from Loba fine, India. Double distilled water was used throughout the experiment. Olsar-A in a tablet dosage form containing OLM and AML were purchased from local commercial sources.

#### Standard solution

##### 1.1. Selection of common solvent

Acetonitrile and glass distilled water was selected as a common solvent for developing spectral characteristics of drug. The selection was made after assessing the solubility of both the drugs in different solvents.

##### 1.2. Preparation of standard drug solution

Standard stock solution containing Olmesartan Medoxomil (OLM) and Amlodipine Besylate (AML) was prepared by dissolving 10 mg of OLM and 10 mg AML separately in 50 ml of Acetonitrile and then final volume of both the solutions was made up to 100 ml with glass distilled water to get stock solution containing 100 µg ml<sup>-1</sup> of OLM and AML in two different 100 ml volumetric flasks.

##### 1.3. Procedure for determining the sampling wavelength for simultaneous analysis

By appropriate dilution of two standard drug solutions with Acetonitrile and glass distilled water, solutions containing 10 µg ml<sup>-1</sup> of OLM and 10 µg ml<sup>-1</sup> of AML were scanned separately in the range of 200-400 nm to determine the wavelength of maximum absorption for both the drugs. OLM and AML showed absorbance maxima at 240 nm and 360 nm respectively.

##### 1.4. Selection of method and wavelength

For estimation of OLM and AML spectrophotometric method employing 265 nm and 360 nm as analytical wavelengths was used. Absoptivity values of both drugs at both the wavelengths were

calculated from absorbance values for the drugs at the selected wavelengths. Simultaneous equations are constructed from the

calculated absorptivity values. The wavelengths were selected from the overlain spectra shown in Fig. 1.

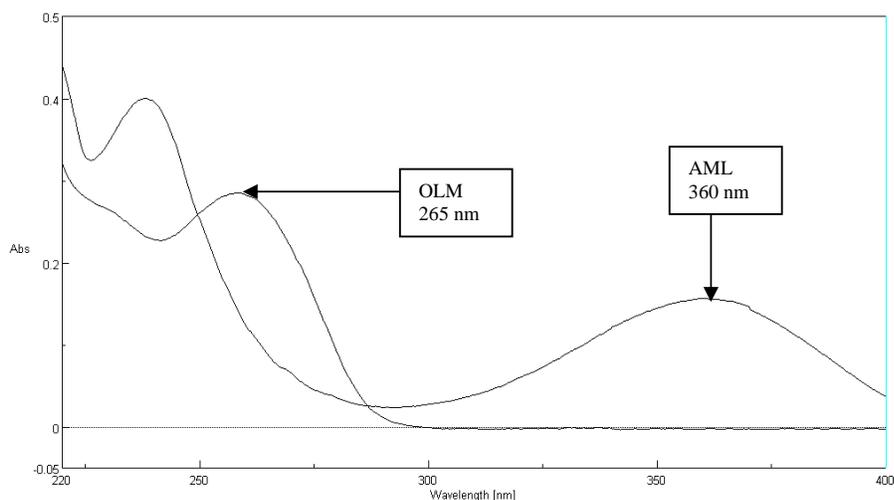


Fig. 1: overlay spectra of OLM and AML

**1.5. Procedure for making mixed standard**

The composition of tablet formulation procured from a local pharmacy was OLM 20 mg and AML 5 mg. The standard stock solutions of OLM and AML were used to prepare mixed standards.

From standard drug solutions nine working standard solutions of OLM 0,4,8,12,16,,20,24,28,32  $\mu\text{g ml}^{-1}$  and AML with concentration of 0,1, 2, 3,4,5,6,7,8  $\mu\text{g ml}^{-1}$  were prepared for both the drugs. The composition of mixed standards is given in Table 1. The overlain spectrum of mixed standards was determined and is given in Fig. 2.

Table 1: Concentration of Mixed Standard

Standard No.	1	2	3	4	5	6	7	8	9
Concentration of AML ( $\mu\text{g ml}^{-1}$ )	0	1	2	3	4	5	6	7	8
Concentration of OLM ( $\mu\text{g ml}^{-1}$ )	32	28	24	20	16	12	8	4	0

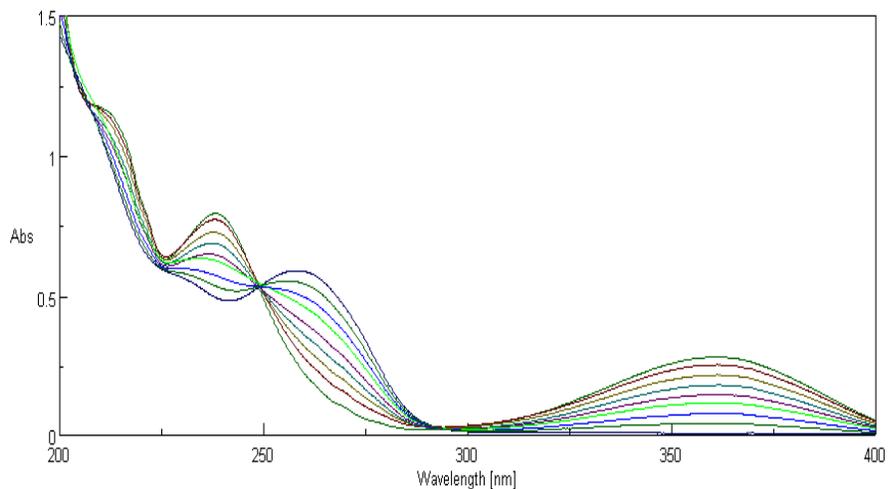


Fig. 2: overlay spectra of mixed standards

**1.6 Derivation of equation**

From the overlain spectrum shown in fig.no.2 the wavelength selected for estimation of AML was 360 nm where OLM has no

absorption and for OLM it was 265 nm, where absorbance of AML is corrected. The quantitative estimation of these drugs was carried out by using following formulae;

$$C_y = A_{360 \text{ nm}} / A(1\%, 1\text{cm})_{360 \text{ nm}} \text{ of AML} \quad (1)$$

$$C_x = A_{265 \text{ nm}} / A(1\%, 1\text{cm})_{265 \text{ nm}} \text{ of OLM} \quad (2)$$

$$CA_{x265 \text{ nm}} = A_{265 \text{ nm}} - A_{y265 \text{ nm}} \quad (3)$$

$$A_{y360 \text{ nm}} = C_y \times A(1\%, 1\text{cm})_{265 \text{ nm}} \text{ of AML} \quad (4)$$

Where,

$C_x$  and  $C_y$  are the concentration (gm/100ml) of OLM and AML respectively.

$A_{360 \text{ nm}}$  and  $A_{265 \text{ nm}}$  are absorbance of mixture at 360 nm and 265 nm respectively.

$CA_{x265 \text{ nm}}$  is corrected absorbance of OLM and

$A_{y360 \text{ nm}}$  is absorbance of AML at 265 nm.

Linear regression data showed a good linear relationship over the concentration of 2 to 32  $\mu\text{g ml}^{-1}$  for OLM as well as 2 to 20  $\mu\text{g ml}^{-1}$  AML. For both the drugs nine point calibration curves were generated shown in fig. 3 and fig. 4. And Results of analysis of laboratory samples are shown in Table. 2:

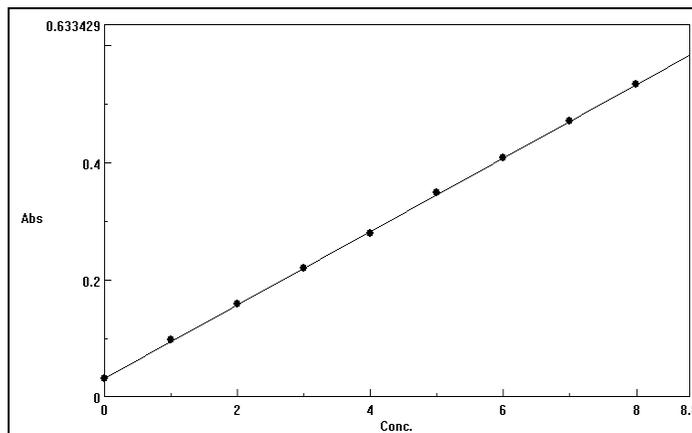


Fig. 3: Calibration curve for OLM

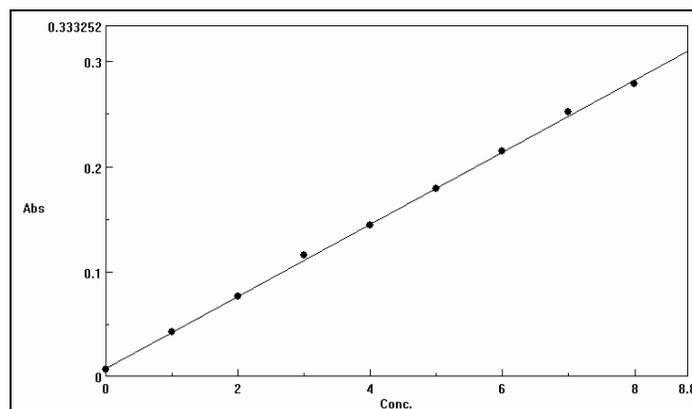


Fig. 4: Calibration curve for AML

Table 2: Results of analysis of laboratory samples

Analyte	% Concentration estimated* (Mean $\pm$ S. D.)	R.S.D.
OLM	100.25 $\pm$ 1.1028	1.1254
AML	99.66 $\pm$ 1.5085	1.6351

\*Average of nine determinations; R.S.D., relative standard deviation

### 1.7. Analysis of tablet formulation

Marketed tablet formulations containing OLM 20 mg and AML 5 mg were analyzed using this method. From the triturate of 20 tablets, an amount equivalent to 20 mg of OLM and 5 mg of AML was weighed and dissolved in 50 ml of acetonitrile in 100 ml volumetric flask. The solution was filtered through Whatmann filter paper no. 41 and then

final volume of the solution was made up to 100 ml with glass distilled water to get a stock solution containing 200  $\mu\text{g ml}^{-1}$  of OLM and 50  $\mu\text{g ml}^{-1}$  AML. After appropriate dilutions, the absorbance was measured and the concentration of each analyte was determined with the equations generated from calibration curve of respective drugs. The statistical data obtained after replicate determinations (n = 9) are shown in Table No. 3.

Table 3: Results of tablet analysis

Analyte	Label claim (mg/tab)	% Label claim estimated* (Mean ± S. D.)	R.S.D.
OLM	20	100.33 ± 1.0892	1.02536
AML	5	100.34 ± 1.5366	1.6257

\*Average of nine determinations; R.S.D., relative standard deviation

#### 1.8. Recovery Studies:

Accuracy of analysis was determined by performing recovery studies by spiking different concentrations of pure drug in the

preanalyzed tablet sample. Results of recovery studies indicated that the method is rapid, accurate and reproducible shown in Table No. 4.

Table 4: Results of recovery study

Analyte	Label claim (mg/tab)	% Recovery estimated* (Mean ± S. D.)	R.S.D.
OLM	20	100.15 ± 0.8905	0.789
AML	5	99.27 ± 0.9785	0.826

\*Average of nine determinations; R.S.D., relative standard deviation

Table 5: Results of repeatability

Analyte	Label claim (mg/tab)	% Label claim estimated* (Mean ± S.D.)	R.S.D.
OLM	20	100.33 ± 1.2256	1.3256
AML	5	101.55 ± 1.3615	1.2568

\*Average of nine determinations; R.S.D., relative standard deviation

Table 6: Results of intraday precision

Time	% Label claim estimated* (Mean ± S.D.)		R.S.D.	
	OLM	AML	OLM	AML
T-1	101.55±1.12652	100.25±1.634	1.1666	1.7258
T-2	100.22±1.9258	101.36±1.5872	0.9896	1.7256
T-3	99.66±1.1667	101.11±1.5891	1.1696	1.8857

\* Average of nine determinations; R.S.D., relative standard deviation.

Table 7: Results of interday precision

Day	% Label claim estimated* (Mean ± S.D.)		R.S.D.	
	OLM	AML	OLM	AML
Day -1	100.86±0.8569	99.45±1.5256	0.8589	1.5245
Day -2	100.34±0.9532	101.25±1.6219	0.9500	1.4156
Day -3	100.96±1.0456	100.85±1.6496	1.0565	1.6326

Average of nine determinations; R.S.D., relative standard deviation

Table 8: Limit of detection and limit of quantitation

LOD ( $\mu\text{g ml}^{-1}$ ) *		LOQ ( $\mu\text{g ml}^{-1}$ ) *	
OLM	AML	OLM	AML
0.0116	0.066	0.0525	0.092

\* Average of six determinations; R.S.D., relative standard deviation.

Table 9: Results of robustness (Analysis using methanol)

Analyte	Label claim (mg/tab)	% Label claim estimated* (Mean ± S.D.)	R.S.D.
OLM	20	99.66 ± 0.9653	0.6989
AML	5	99.50 ± 1.5625	1.6545

\* Average of nine determinations; R.S.D., relative standard deviation

### 1.9. Method Validation

The method was validated according to ICH Q2B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for the analyte<sup>33</sup>. Results are shown in **Table No. 5 to 9**.

### RESULTS AND DISCUSSION

An attempt has been made to develop a fast, sensitive, precise, reproducible and economical analytical method for simultaneous estimation of OLM and AML in their combined dosage form. In this method drug obeys Beer's law in the concentration range of 2-20  $\mu\text{g ml}^{-1}$  and 2-32  $\mu\text{g ml}^{-1}$  for OLM and AML respectively.

The absorption additivity study was carried out to see whether the drugs in mixture show additivity or not. It was observed that both the drugs showed the additivity of absorbance at selected wavelength indicating that both the drugs do not interact with each other in the solvent system used for absorption correction method. Literature survey has revealed that few methods are reported for

estimation of Olmesartan medoxomil and numbers of methods are reported for estimation of Amlodipine besylate individually as well as in combination with another drugs but no method is reported for simultaneous estimation by absorption correction in fixed dose combination pharmaceutical formulation.

The proposed method for simultaneous estimation of OLM and AML utilizes the spectrum mode of analysis of Jasco V-530 spectrophotometer. The method requires nine mixed standard solutions involving scanning between 200 to 400 nm. Sampling wavelengths based upon the direct UV spectroscopic data. For OLM, the interference due to AML was eliminated by taking the absorbance at 265 nm whereas quantification of AML was achieved by measurement of absorbance at 360 nm because at this wavelength no interference from OLM was observed. Linear regression data showed a good linear relationship over the concentration of 2 to 32  $\mu\text{g ml}^{-1}$  for OLM as well as 2 to 20  $\mu\text{g ml}^{-1}$  AML. For both the drugs nine point calibration curves were generated. The optical characteristics are shown in table **No.10**.

**Table 10: Optical Characteristics of developed methods**

Parameters	OLM	AML
$\lambda_{\text{max}}$	265 nm	360 nm
Beers law limit ( $\mu\text{g ml}^{-1}$ )	2-32	2-20
Regression Equation data:		
Slope	0.0156	0.0081
Intercept	0.0326	0.0343
Correlation coefficient	0.99925	0.9996

$Y = A + B \cdot C$ , Where C is the concentration in  $\mu\text{g ml}^{-1}$  and Y is absorbance unit.

Interday and intra day studies showed high degree of repeatability of an analytical method under normal operating conditions. Interday and intraday studies showed repeatability of the method. Results of tablet analysis in the range of 98.15 to 100.15% and 99.67 to 100.88% for OLM and AML, which indicate repeatability of the method. Lower limit of detection for OLM and AML was found to be 0.0116  $\mu\text{g ml}^{-1}$  and 0.066  $\mu\text{g ml}^{-1}$  and Limit of quantitation was found to be 0.0525  $\mu\text{g ml}^{-1}$  and 0.092  $\mu\text{g ml}^{-1}$  Standard deviation for tablet analysis by using methanol was ranging from 99.15 to 100.05 % for OLM and 99.86 to 100.58 % for AML.

Recoveries obtained for the drugs do not differ significantly from 100% showed that there was no interference from the common excipients used in the tablet formulation indicating accuracy and reliability of the method. Precision for tablet analysis was determined by analysis of tablets containing OLM and AML which proves the ability of the method to remain unaffected by small but deliberate changes in the conditions of analysis.

### CONCLUSION

The proposed method for simultaneous estimation of Olmesartan medoxomil and Amlodipine besylate in their combined dosage form are quite accurate, precise, yield reproducible result and rugged. Moreover the method is economic, simple and rapid, hence can be employed for routine analysis in quality control laboratories.

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#### List of non-standard abbreviations used

- **rpm**: revolutions per minute
- **OLM**: Olmesartan Medoxomil
- **AML**: Amlodipine Besylate
- **Abs**: Absorbance
- **Conc.**: Concentration
- **S.D.**: Standard Deviation
- **R.S.D.**: Relative Standard Deviation.
- **n**: Number of times analysis is repeated