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Simultaneous estimation of Amlodipine besylate and Olmesartan medoxomil by First Order Derivative Spectroscopy from Tablet

Pournima S. Patil*[1], Pramodini D. Kulkarni [1] ,Mallappa S. Burkul[1], Harinath N.More[2], Sachin A. Pishawikar[2]

[1]KCT's Krishna College of Pharmacy, Malkapur, Karad- 415110(M.S.), India.

[2]Bharati Vidyapeeth College of Pharmacy, Morewadi, Kolhapur-416013 (M.S.) India.

*Corres.author : poournima6@gmail.com
Phone Number: 9890818046, Fax: +91 02164 242170*

Abstract: A Simultaneous determination of Olmesartan Medoxomil (OLM) and Amlodipine Besylate(AML) by first order derivative spectrophotometric method has been developed in combined dosage form. The method is based on measurement of absorbance at 226 nm as wavelengths for quantification of OLM where no interference due to AML was observed similarly absorbance at 241 nm is selected for quantification of AML, where OLM did not interfere with the estimation of AML. The system obeyed Beers law over the concentration of 2 to 32 µg ml $^{-1}$ for OLM and 2 to 20 µg ml $^{-1}$ for AML.respectively.

The proposed method was validated and can be used for analysis of combined dosage tablet formulation containing Olmesartan medoxomil and amlodipine besylate. Interday and intraday studies showed repeatability of the method.

Key words: Amlodipine besylate, Olmesartan medoxomil, Spectroscopy.

Introduction:

Olmesartan Medoxomil is a prodrug and is hydrolyzed to the active olmesartan during absorption from the gastrointestinal tract.olmesartan is a selective angiotensin II Receptor antagonist. It is a chemically,2,3-dihydroxy-2-butenyl 4-(1 hydroxy-1 methyl ethyl)-2-propyl-1-[p-(o-1Htetrazol-5-ylphenyl)benzyl]imidazole-5-carboxylate,cyclic-2,3carbonate.

Amlodipine besylate is one of the long acting calcium channel blocker used as an also antihypertensive agent. It is chemically [3-ethyl-5 methyl (4 RS)-2-[(2 Amino ethoxy)methyl]-4-(2 chlorophenyl)-methyl-1-dihydro pyridine-3,5-dicarboxylate benzenesulfonate.

Several analytical methods have been reported for the determination of olmesartan medoxomil in biological fluids includes LC-MS-MS ,degradation product HPLC[(1-11)] A number of method have been reported for estimation of AML individually or in combination with other drugs.[(12-28).]However, there is no analytical method reported for the simultaneous determination of AML and OLM in a combined dosage form by derivative spectroscopy. Data obtained from interday and intraday studies showed high degree of repeatability of an analytical method under normal operational conditions. Result of analysis of tablet formulation showed % relative standard deviation values in the range of 0.70 to 1.012 for OLM and 0.80 to 0.98 % for AML, which indicates repeatability of the method. The results indicated excellent recoveries ranging from 99.60 to 100.83 for OLM and 99 to 101.68 % for AML.Recoveries obtained for the two drugs do not differ significantly from 100 %, which showed that there was no interference from common excipients used

in the formulation indicating accuracy and reliability of the method. While limit of quantitation for OLM and AML was found to be 0.0480 and 0.45 resp. %.S.D. value for tablet analysis by using methanol was found to be ranging from 98.5 to 102.2 for OLM and 99.7 to 102.4 % for AML. Which proves the ability of the method to remain unaffected by small but deliberate changes in conditions of analysis.

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. 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.

Experimental:

Materials:

Instrument:

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 Chemicals:

Olmesartan Medoxomil and amlodipine besylate were obtained as a gift sample from Cipla, Vapi, India. All chemicals were analytical grade obtained from SD fine chemicals. Water purified by glass distillation apparatus.Olsar-A in a tablet dosage form containing OLM and AML were purchased from local commercial sources.

1.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 and reagents:

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, sonicated for 20 minutes 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^{-1} of OLM and AML in two different 100 ml volumetric flasks.

1.3. Procedure for Determining the Sampling Wavelength for Simultaneous Analysis:

OLM and AML (10 µg ml^{-1} each) were scanned separately in a wavelength range of 200-400 nm against acetonitrile: glass distilled water (50:50) blank. The first derivative spectra were obtained by instrumental electronic differentiation in the range of 200 to 300 nm. A signal at 1 D_{226} of first derivative spectrum was selected for quantification of OLM where no interference due to AML was observed similarly a signal at 1 D_{241} was selected for quantification of AML, where OLM did not interfere with the estimation of AML. A first derivative overlain spectrum of OLM and AML is shown in Fig. No. 1.

Fig. No. 1. First derivative overlain spectra of OLM and AML

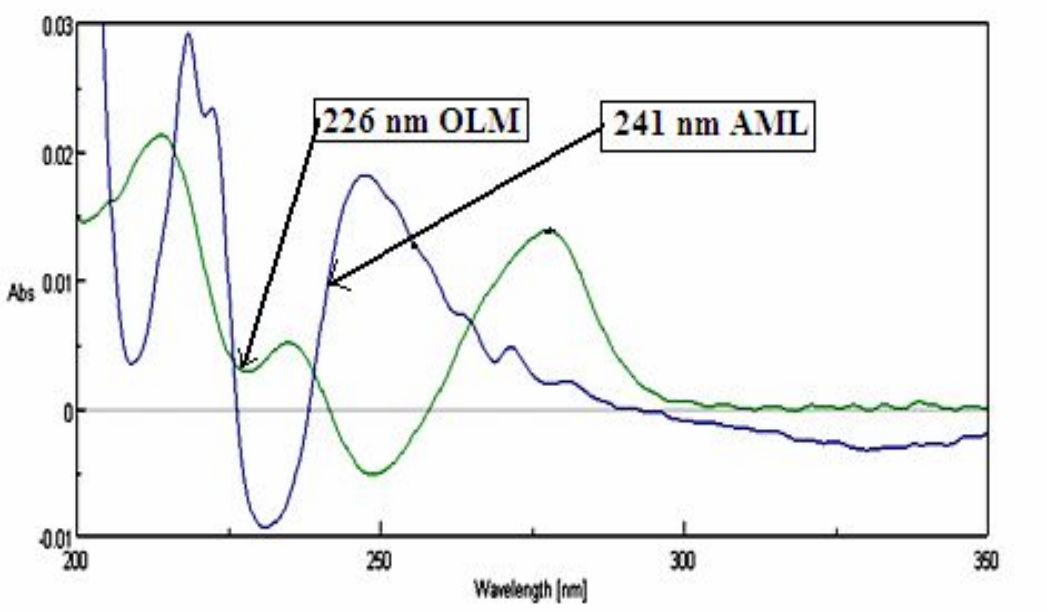
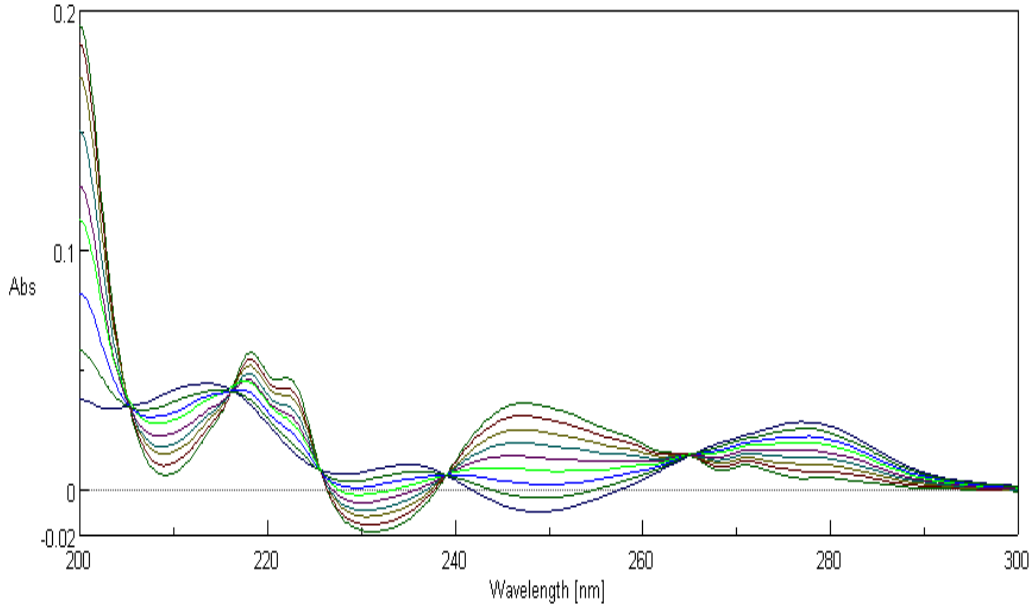


Fig. No. 2. First derivative overlain spectra of mixed standard.



The standard stock solutions of OLM and AML were used to prepare mixed standards. From standard drug solutions nine working standard solutions of OLM 0,8,12,16,20,24,28,32,36, µg ml^{-1} and[1] AML with concentration of 0,1,2,3,4,5,6,7,8 µg ml^{-1} were prepared for both the drugs. The composition of mixed standards is given in Table No. 1**.** The overlain spectrum of mixed standards was determined and is given in Fig. No. 2.

1.5. Procedure for Plotting Calibration Curve:

The above nine mixed standard solutions were scanned in the selected analytical wavelengths and the calibration curve for both the drugs was constructed. Calibration curve for OLM was plotted against concentration by taking absorbance at 1 D_{226} from the spectra of mixed standards while calibration curve for AML was plotted against concentration using absorbances at 1 D_{241}. AML obeyed Beer's law in the concentration range of 2-20 µg ml^{-1}.and OLM obeyed Beer's law in the concentration range of 2-32 µg ml^{-1} By using quantitative modes of instrument slope, intercept and correlation coefficient values for calibration curve was obtained for both the drugs. For OLM, the concentration in sample solution was calculated by using formula Abs = A + B * C, where A = 0.0028, B = 0.0001, C = concentration of OLM and correlation coefficient for OLM was 0.999165. For AML, the concentration in sample solution was calculated by using formula Abs = A + B * C, where A = 0.0014, B = 0.0019, C = concentration of AML and correlation coefficient fro AML was 0.999536. Calibration curves absorbances are shown in Table No. 2 and Table No. 3. Optical characteristics are shown in Table No. 4. Results of analysis of laboratory samples are shown in Table No. 5.

Table No. 1. Concentration of Mixed Standard:

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

Table No. 2. Absorbance values for Calibration curve of OLM:

Sr. No.	Concentration (µg ml^{-1})	Absorbance
1.	4	0.00311646
2.	8	0.00340603
3.	12	0.00382601
4.	16	0.0041180
5.	20	0.0044569
6.	24	0.0047533
7.	28	0.0050124
8.	32	0.00535313

Table No. 3. Absorbance values for Calibration curve of AML:

Sr. No.	Concentration (µg ml^{-1})	Absorbance
1.	1	0.00330048
2.	2	0.0052834
3.	3	0.00731675
4.	4	0.00929801
5.	5	0.0111753
6.	6	0.0130028
7.	7	0.0149827
8.	8	0.01649

Table No. 4. Optical Characteristics:

Parameters	OLM	AML
λ_{max}	226 nm	241 nm
Beers law limit (µg ml^{-1})	2-32	2-20
Regression Equation data:		
Slope	0.0001	0.0019
Intercept	0.0028	0.0014
Correlation coefficient	0.999165	0.999536

Y= A+B*C, Where C is the concentration in µg ml^{-1} and Y is absorbance unit.

Table. No. 5. Results of analysis of laboratory samples:

Analyte	% Concentration estimated* (Mean ± S. D.)	R.S.D.
OLM	100.07 ± 1.1971	1.1908
AML	101.08 ± 1.06	1.05

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

1.6. 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 µg ml^{-} of OLM and 100 µg ml^{-} AML. After appropriate dilutions, the absorbances were 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. 6.

Table. No. 6. Results of tablet analysis:

Analyte	Label claim (mg/tab)	% Label claim estimated* (Mean ± S. D.)	R.S.D.
OLM	20	100.37 ± 0.8551	0.8519
AML	5	100.94 ± 0.8847	0.8764

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

Table. No. 7. Results of recovery study:

Analyte	Label claim (mg/tab)	% Recovery estimated* (Mean ± S. D.)	R.S.D.
OLM	20	100.20 ± 0.3815	0.3807
AML	5	100.24 ± 0.9129	0.8764

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

1.7. 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.7.

1.8. Method Validation:

The method was validated according to ICH Q2B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision, robustness and accuracy for the analyte (29). Accuracy and specificity of analysis was determined by performing recovery studies by spiking different concentrations of pure drug in the preanalyzed tablet sample. Results of validation parameters are reported in Table No. 8 to 12.

Table. No. 8. Results of repeatability:

Analyte	Label claim (mg/tab)	% Label claim estimated* (Mean ± S. D.)	R.S.D.
OLM	20	99.69 ± 1.4936	1.4981
AML	5	100.12 ± 0.3227	0.3223

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

Table. No. 9. Results of Intraday Precision:

Time	% Label claim estimated* (Mean ± S.D.)		R.S.D.	
	OLM	**AML**	**OLM**	**AML**
T-1	100.14±1.1610	100.97±0.9583	1.159	0.9490
T-2	100.34±1.6654	101.17±0.9728	1.65	0.9616
T-3	100.19±1.088	101.05±1.057	1.086	1.0467

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

Table. No. 10. Results of Interday Precision:

Day	% Label claim estimated* (Mean ± S.D.)		R.S.D.	
	OLM	**AML**	**OLM**	**AML**
Day -1	100.43±1.213	100.99± 1.04	1.208	1.036
Day -2	100.41±1.021	101.12±0.9687	1.017	0.9579
Day -3	100.53±1.1693	101.17±0.962	1.1631	0.9515

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

Table. No. 11. Limit of Detection and Limit of Quantitation:

LOD (µg ml $^{-1}$) *		LOQ (µg ml $^{-1}$) *	
OLM	**AML**	**OLM**	**AML**
0.0166	0.2200	0.0480	0.45

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

Table. No. 12. Results of robustness (Analysis using methanol)

Analyte	Label claim (mg/tab)	% Label claim estimated* (Mean ± S. D.)	R.S.D.
OLM	20	100.34 ± 1.1385	1.1346
AML	5	100.86 ± 1.0793	1.0700

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

1.9. Results and Discussion:

The zero-order spectra of pure drugs were found to be overlapping making their simultaneous determination difficult. The first derivative spectrophotometric method was considered to be ideal to facilitate their quantitative determination. It was observed during initial study that first derivative spectra have ideal zero-crossing points for the estimation of OLM and AML in their combined dosage form. The method utilizes nine mixed standard solutions which were scanned in a wavelength range of 200-400 nm against acetonitrile: water (50:50) as blank. Their first derivative spectra were obtained by instrumental electronic differentiation in the range of 200-300 nm. There was no interference of OLM at a signal 1 D_{241} of first derivative spectrum of AML, thus this wavelength was selected for quantification of AML while a no interference of AML at a signal 1 D_{226} of first derivative spectrum of OLM, thus this wavelength was selected for quantification of OLM.

Linear regression data Linear regression data showed a good linear relationship over a concentration range of 2-20 µg ml^{-1} for AML and 2-32 µg ml^{-1} for OLM. For both the drugs eight point calibration curves were generated. Data obtained from Interday and intraday studies showed high degree of repeatability of an analytical method under normal operational conditions. Result of analysis of tablet formulation showed % relative standard deviation values in the range of 0.70 to 1.012 for OLM and 0.80 to 0.98 % for AML, which indicates repeatability of the method. The results indicated excellent recoveries ranging from 99.60 to 100.83 for OLM and 99 to 101.68 % for AML.Recoveries obtained for the two drugs do not differ significantly from 100 %, which showed that there was no interference from common excipients used in the formulation indicating accuracy and reliability of the method.

While limit of quantitation for OLM and AML was found to be 0.0480 and 0.45 resp. %.S.D. value for tablet analysis by using methanol was found to be ranging from 98.5 to 102.2 for OLM and 99.7 to 102.4 % for AML. Which proves the ability of the method to remain unaffected by small but deliberate changes in conditions of analysis.

1.10. Discussion:

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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