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Simulteneous determination of Olmesartan medoxomil and Amlodipine besylate from Tablet Formulation by Multiwavelength Method

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Abstract: Spectrophotometric method for determination of Olmesartan medoxomil and amlodipine besylate in pure form or in pharmaceutical formulation by multiwavelength method was described. The method requires nine standard solutions scanning between 265, 324 and 360 nm as wavelengths. For OLM, the interference due to AML was eliminated by absorbance difference at 265 and 324 nm whereas quantification of AML 360 nm.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. Interday and intraday studies showed repeatability of the method. Results of tablet analysis in the range of 99.29 to 101.93% and 98.77 to 101.83% for OLM and AML, which indicate repeatability of the method.
The recoveries from 97.99 to 101.23 % and 98.46 to 102.31 % 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. Lower limit of detection for OLM and AML was found to be 0.0116 µg ml $^{-1}$ and 0.049 µg ml $^{-1}$. Limit of quantitation was found to be 0.0335 µg ml $^{-1}$ and 0.093 µg ml $^{-1}$ Standard deviation for tablet analysis by using methanol was ranging from 99.1 to 101.9 % for OLM and 98.8 to 101.98 % for AML. Which proves the ability of the method remains unaffected by small but deliberate changes in the conditions of analysis.
Key Words: Amlodipine besylate, Olmesartan medoxomil, Specroscopy.

Introduction:

Combinations of two or more drugs in the pharmaceutical dosage forms are very much useful in multiple therapies. 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[1]. 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[2]. 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.

Amlodipine Besylate and Olmesartan Medoxomil is recently introduced in the market as combined tablet dosage form which is widely used in the treatment of hypertension[3, 4]. There is no method reported for simultaneous estimation of OLM [5-8] and AML [9-16] from dosage forms by multiwavelength method.. 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.

Experimental:

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^{-1} 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^{-1} of OLM and 10 µg ml^{-1} 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 258 nm and 317 nm respectively. Individual and overlain spectra for both the drugs are shown in Fig. No. 1 to 2.

1.4. Selection of Method and Wavelength:

For estimation of OLM, multi-wavelength spectrophotometric method employing 265 nm and 324 nm as analytical wavelengths was used; the two wavelengths were chosen to eliminate interference of AML at the sampling wavelength of OLM. For estimation of AML, 360 nm was selected as the analytical wavelength, as OLM shows no absorption at this wavelength. In the multi-wavelength method developed for simultaneous estimation of OLM and AML, the wavelengths were selected from the overlain spectra shown in Fig. No.1.

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 µg ml^{-1} and 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. 2. The overlain spectrum of mixed standards was determined and is given in Fig. No. 2.

1.6 Procedure for Plotting Calibration Curve:

The above nine mixed standard solutions were scanned in the wavelength range of 200-400 nm and the calibration curve for both the drugs was constructed. Calibration curve for OLM was plotted by recording absorbance difference at the two selected wavelengths (265 nm and 324 nm) against the concentration of drug in nine mixed standards. Similarly calibration curve for AML was plotted by recording absorbance at the selected wavelength (360 nm) against the concentration of drug in nine mixed standards. 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 were obtained for both the drugs. For OLM, the concentration in sample solution was calculated by using formula Abs = A + B * C, where A = 0.0326 B = 0.0156, C = concentration of OLM and correlation coefficient for OLM was 0.999921 For AML, the concentration in sample solution was calculated by

using formula Abs = A + B * C, where A = 0.0081, B = 0.0343, C = concentration of AML and correlation coefficient fro AML was 0.9996. Calibration curves are shown in Fig. No. 1 and Fig. No. 2 Optical characteristics are shown in Table No. 1. Results of analysis of laboratory samples are shown in Table No. 5.

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 µg ml^{-1} of OLM and 50 µg 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. 8.

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. 7.

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 [17]. Results are shown in Table No. 8 to 10.

Table 1: Optical Characteristics of Developed Methods

Parameters	OLM	AML
λ_{max}	265 nm	360 nm
Beers law limit (µg ml^{-1})	2-32	2-20
Regression Equation data:		
Slope	0.0156	0.0081
Intercept	0.0326	0.0343
Correlation coefficient	0.999921	0.9996

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

Table No. 2. 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. 3. Absorbance values for Calibration curve of OLM:

Sr. No.	Concentration (µg ml^{-1})	Absorbance
1.	0	0.032085
2.	4	0.096634
3.	8	0.1586195
4.	12	0.2194909
5.	16	0.2783632
6.	20	0.3488899
7.	24	0.4069875
8.	28	0.4702261
9	32	0.5332053

Table No. 4. Absorbance values for Calibration curve of AML:

Sr. No.	Concentration (µg ml^{-1})	Absorbance
1.	0	0.0238
2.	1	0.0448
3.	2	0.0652
4.	3	0.0852
5.	4	0.1062
6.	5	0.1279
7.	6	0.1487
8.	7	0.1668

Fig. No. 1. Overlain spectra of OLM and AML.

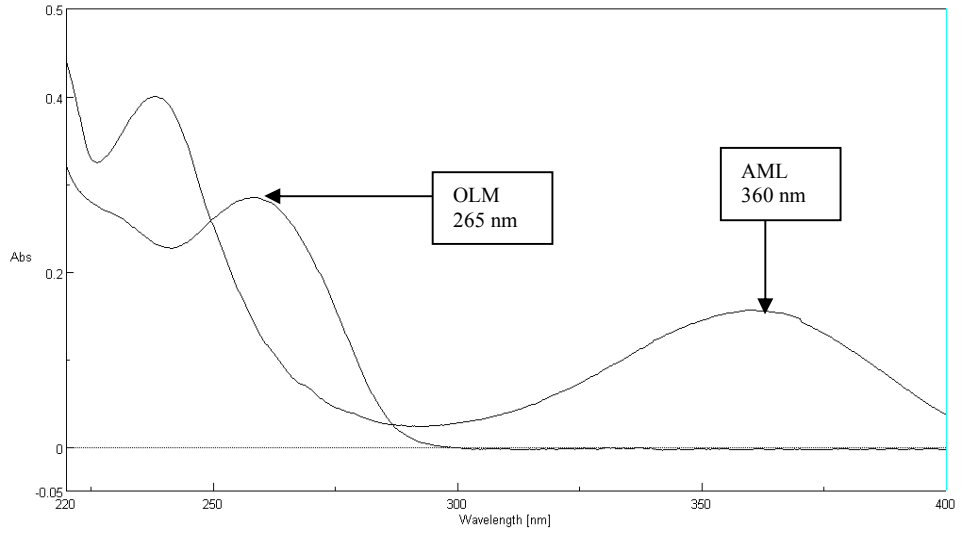

Fig. No. 2. Overlain spectra of Mixed Standards.

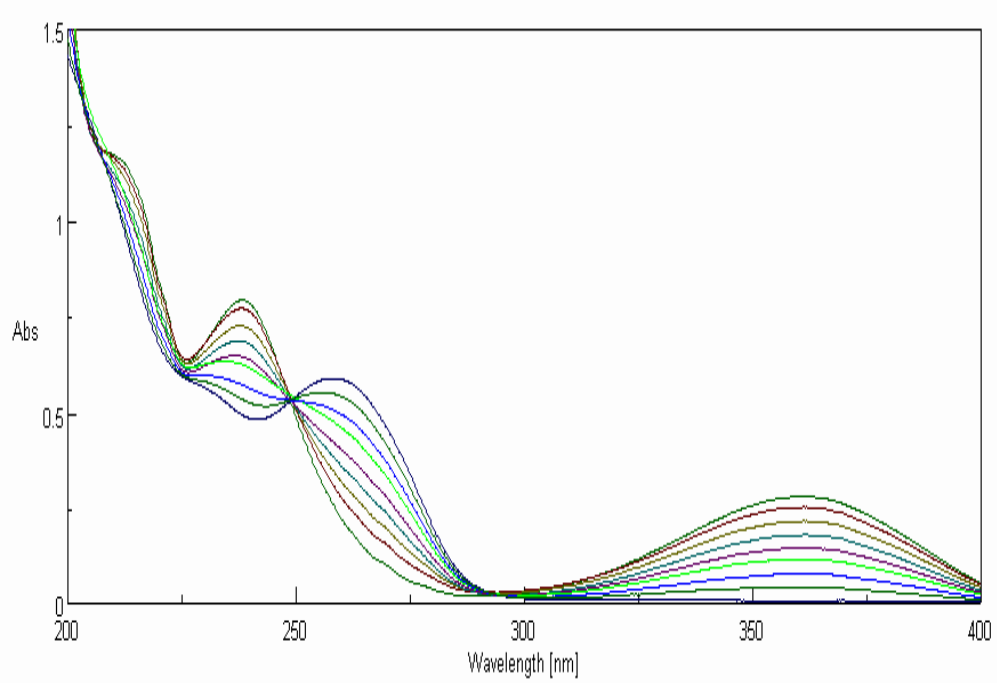

Table. No. 5. Results of analysis of laboratory samples:

Analyte	% Concentration estimated* (Mean ± S. D.)	R.S.D.
OLM	100.22 ± 1.1605	1.1579
AML	100.45 ± 1.5883	1.5811

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

Table. No. 6. Results of tablet analysis:

Analyte	Label claim (mg/tab)	% Label claim estimated* (Mean ± S. D.)	R.S.D.
OLM	20	100.53 ± 1.04342	1.03786
AML	5	100.32 ± 1.4854	1.4805

***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	99.85 ± 0.6087	0.6095
AML	5	100.28 ± 0.9209	0.9183

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

Table. No. 8. Results of repeatability

Analyte	Label claim (mg/tab)	% Label claim estimated* (Mean ± S.D.)	R.S.D.
OLM	20	100.73 ± 1.28187	1.27247
AML	5	99.50 ± 1.3039	1.3103

***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	100.34±1.1756	100.25±1.7125	1.1658	1.7081
T-2	100.3±0.9669	100.30±1.6522	0.9640	1.6471
T-3	100.47±1.1787	100.11±1.5595	1.1731	1.5577

***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	99.5±0.8692	100.11±1.5201	0.8672	1.5184
Day -2	100.34±0.9532	100.18±1.5219	0.9500	1.5191
Day -3	100.46±1.0608	100.05±1.3463	1.0560	1.3456

***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
0.0116	0.049	0.0335	0.093

*** 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	100.37 ± 0.9750	0.9713
AML	5	100.45 ± 1.5525	1.5454

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

1.10. Results and Discussion:

Now a day's combination therapy is commonly used clinically and analyst is required to develop the method for their analysis. The numbers of techniques are available for estimation of individual drugs or in combination. Market survey has revealed that the combination of Olmesartan medoxomil and Amlodipine besylate is recently introduced in the market and is widely used in the treatment of Hypertension. 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 but no method is reported for simultaneous estimation of Olmesartan medoxomil and Amlodipine besylate 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 265, 324 and 360 nm as sampling wavelengths based upon the direct UV spectroscopic data. There is no wavelength where OLM can be accurately quantified without substantial interference of AML. For OLM, the interference due to AML was eliminated by taking the absorbance difference at 265 and 324 nm whereas quantification of AML was achieved by measurement of absorbance directly 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 µg ml $^{-1}$ for OLM as well as 2 to 20 µg ml $^{-1}$AML. For both the drugs nine point calibration curves were generated. Interday and intra day studies showed high degree of repeatability of an analytical method under normal operating conditions. Results of tablet analysis showed standard deviation in the range of 99.29 to 101.93% and from 98.77 to 101.83% for OLM and AML respectively, which indicate repeatability of the method.

The accuracy of the method was determined by investigating the recovery of the two drugs using spiked concentrations of the standard drug. The results indicated excellent recoveries ranging from 97.99 to 101.23 % and 98.46 to 102.31 % for OLM and AML respectively. 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.

Lower limit of detection for OLM and AML was found to be 0.0116 µg ml $^{-1}$ and 0.049 µg ml $^{-1}$ respectively. Limit of quantitation was found to be 0.0335 µg ml $^{-1}$ and 0.093 µg ml $^{-1}$ respectively.S.D. value for tablet analysis by using methanol was found to be ranging from 99.1 to 101.9 % for OLM and 98.8 to 101.98 % for AML. which proves the ability of the method to remain unaffected by small but deliberate changes in the conditions of analysis.

1.11. 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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