

DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF AMLODIPINE BESYLATE AND OLMESARTAN MEDOXOMIL IN COMBINED DOSAGE FORM

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ABSTRACT

Two simple, accurate, rapid and precise UV spectrophotometric methods have been developed for simultaneous estimation of Amlodipine Besylate and Olmesartan Medoximil in combined tablet formulation. Amlodipine Besylate and Olmesartan Medoximil at their respective λ_{max} 237nm and 251 nm shows linearity in a concentration range of 2.5-12.5 $\mu\text{g/ml}$ and 5-25 $\mu\text{g/ml}$ respectively. The methods employed were (A) Absorbance ratio and (B) Dual wavelength. Method-A involves measurement of absorbance at 298.0nm (iso-absorptive point) and 237.0 nm (λ_{max} of Amlodipine Besylate). Method-B involves The wavelengths selected for determination of Amlodipine Besylate were 232 nm and 241.6 nm, whereas the wavelengths selected for determination of Olmesartan Medoximil were 244 nm and 255.4 nm. The linearity for Amlodipine Besylate and Olmesartan Medoximil were found over the concentration range of 2.5-12.5 $\mu\text{g/ml}$ and 5-25 $\mu\text{g/ml}$ respectively. Both the methods were found to be rapid, specific, precise and accurate, can be successfully applied for the routine analysis of Amlodipine Besylate and Olmesartan Medoximil in bulk, and combined dosage form without any interference by the excipients. The method was validated according to ICH guidelines.

Keywords: Amlodipine Besylate, Olmesartan Medoximil, Absorbance ratio method, Dual wavelength method.

INTRODUCTION

Amlodipine Besylate (AMLO), chemically, [3-ethyl-5-methyl(4RS)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-methyl-1-dihydropyridine-3, 5-dicarboxylate benzenesulfonate¹ (fig.-1), is a long acting calcium channel blocker which is used as an antihypertensive agent. Amlodipine is official in I.P., B.P., U.S.P. Amlodipine acts by relaxing the smooth muscle in the arterial wall, decreasing total peripheral resistance, hence reducing blood pressure; in angina it increases blood flow to the heart muscle².

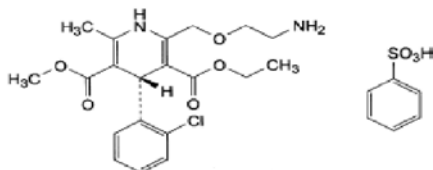


Fig. 1: Structure of Amlodipine Besylate

Olmesartan Medoximil (OLME) 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 which is used as an antihypertensive agent³. Olmesartan works by blocking the binding of angiotensin II to the AT1 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.⁴

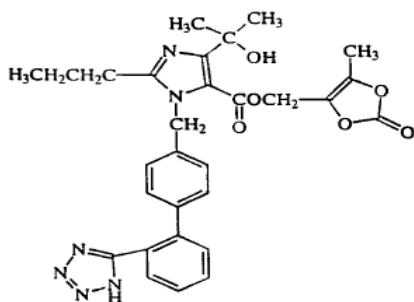


Fig. 2: Structure of olmesartan medoximil

Literature survey revealed that a number of methods have been reported for estimation of AMLO and OLME individually or in combination with other drugs⁵⁻¹⁶. However, there is no Dual wavelength and Absorbance ratio reported for the simultaneous estimation of AMLO and OLME in a combined dosage formulation. Present work describes two simple, accurate, reproducible, rapid and economical methods for simultaneous estimation of Amlodipine Besylate and Olmesartan Medoximil in tablet formulation.

MATERIALS AND METHOD

Instrument

A double-beam Shimadzu UV/Vis spectrophotometer, 1800 with spectral bandwidth of 2 nm, wavelength accuracy of ± 0.5 nm and a pair of 1-cm matched quartz cells, was used to measure absorbance of the resulting solution. All weighing were done on electronic balance (Model Shimadzu AUW-220D), Ultrasonicator model 5.5L150H were used.

Chemicals and reagents

AR grade of methanol was used, purchased from Astron Chemicals LTD.

Standard gift sample of Amlodipine Besylate and olmesartan medoximil was obtained from Cadila pharma ltd., Dholka, Gujarat, India.

Amlodipine Besylate and Olmesartan Medoximil combination tablets (OLMESAR, 5 mg Amlodipine Besylate and 20 mg Olmesartan Medoximil; is manufactured by Macleods Pharmaceuticals Ltd., Himachal Pradesh, India).

Preparation of standard stock solution (100 $\mu\text{g/ml}$)

The stock solution (100 $\mu\text{g/ml}$) of AMLO and OLME were prepared separately by dissolving accurately about 10 mg of drug in Methanol and the volume was made up to 100 ml with Methanol to prepare standard stock solution (100 $\mu\text{g/ml}$).

Preparation of Calibration curve of AMLO and OLME

The standard stock solution (100 $\mu\text{g/ml}$) of AMLO and OLME were further diluted with methanol to obtain the final concentration 2.5, 5, 7.5, 10, 12.5 $\mu\text{g/ml}$ and 5, 10, 15, 20, 25 $\mu\text{g/ml}$ respectively. The standard solution of both drug were scanned in the spectrum mode from 200 nm to 400 nm against solvent methanol and spectra was recorded. λ_{max} of AMLO and OLME was found 237.0 nm (Fig.3) & 252.0 nm (Fig.4) respectively.

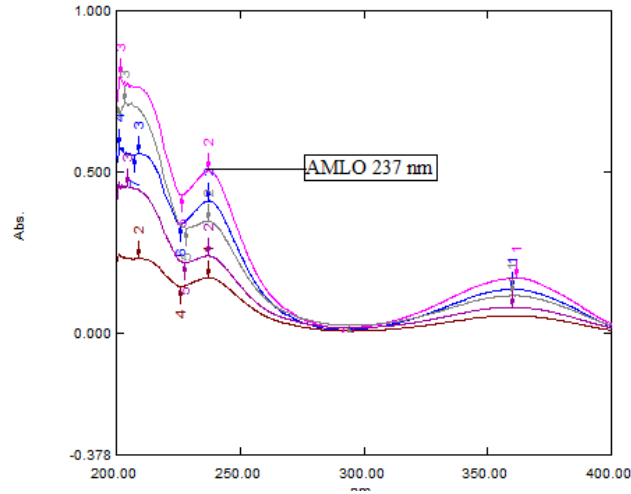


Fig. 3: Overlay spectra of Amlodipine Besylate at 237.0 nm.

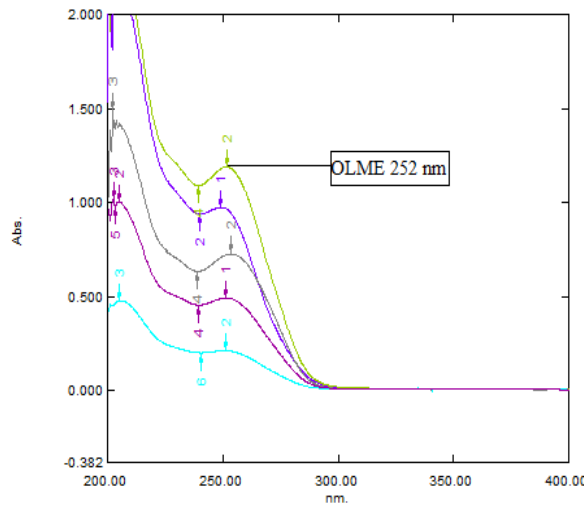


Fig. 4: Overlay spectra of Olmesartan medoximil at 252.0 nm.

Procedure

Method A (Absorbance ratio method)

It uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the λ-max of one of the two components. From the overlay spectra of two drugs, it is evident that AMLO and OLME show an isoabsorptive point at 298.0 nm. The second wavelength used is 237 nm, which is the λ-max of AMLO. Five working standard solutions having concentration 2.5, 5, 7.5, 10, 12.5µg/ml for AMLO and 5, 10, 15, 20, 25 µg/ml for OLME were prepared in methanol and the absorbances at 298.0 nm (isoabsorptive point) and 237.0 nm (λ-max of AMLO) were measured and absorptivity coefficients were calculated using calibration curve.

Absorptivity = Absorbance/ Concentration of that component in gm/100 ml.

The concentration of two drugs in the mixture can be calculated using following equations.

$$C_A = [(Q_M - Q_0) / (Q_A - Q_0)] \times A_1/aX_1 \dots\dots\dots (1)$$

$$C_0 = (A_1/aX_1) - C_A \dots\dots\dots (2)$$

Where, A₁ and A₂ are absorbances of mixture at 298.0 nm and 237.0 nm;

aX₁ and aY₁ are absorptivities of AMLO and OLME at 298.0 nm;

aX₂ and aY₂ are absorptivities of AMLO and OLME respectively at 237.0 nm;

$$Q_M = A_2 / A_1,$$

$$Q_A = aX_2 / aX_1 \text{ and}$$

$$Q_0 = aY_2 / aY_1.$$

Validation data for this method are given in Table-1.

Method B (Dual wavelength)

The solution of AMLO and OLME were prepared separately in methanol having concentration of 2.5-12.5 µg/ml and 5-25µg/ml . They were scanned in the wavelength range of 200 - 400 nm. From the overlain spectra of both drugs, four wavelengths 232nm, 241.6nm, 244 nm and 255.4 nm were selected for quantitation of both the drugs by proposed dual wavelength spectrophotometric method. The quantitative determination of AMLO is carried out by measuring the absorbance value at 232nm and 241.6 nm, and the difference between 232nm and 241.6 nm is directly proportional to concentration of OLME in the mixture, whereas determination of OLME is carried out by measuring the absorbance value at 244 nm and 255.4 nm and the difference between 244 nm and 255.4 nm is directly proportional to concentration of AMLO in the mixture.

Validation data for this method are given in Table-2.

Procedure for the Analysis of Tablet formulation

Twenty tablets containing label claim of 5mg of AMLO and 20 mg of OLME were weighed and finely powdered .The average weight was calculated. Weight of the powder equivalent to 5 mg AMLO was

accurately weighed, transferred into a 25 ml flask, dissolved in methanol and sonicated for 20 min. The volume is adjusted up to the mark with methanol. The solution was then filtered through Whatman filter paper no. 41.

The solution was suitably diluted with methanol to get a final concentration of 2.5 µg/ml of AMLO and 10 µg/ml of OLME.

Concentrations of both AMLO and OLME were determined by measuring the absorbance of the sample at 298.0 nm (isoabsorptive point-Fig. 5) and 237.0 nm (λ -max of AMLO-Fig.6) respectively, and ratios of absorbance were calculated, i.e. A_2/A_1 . (method-A) and at 241.6&232.0 nm(Fig.7) and 244.0-255.4 nm(Fig.8) (method B) in the spectrum mode and values were substituted in the respective formula to obtain concentrations. Results of the tablet analysis were analyzed against the calibration curve in quantitation mode (Table

3). The analysis procedure was repeated three times with tablet formulation. Overlain spectra for both methods (Fig. 9 and Fig. 10). Recovery studies was performed (Table-4)

RESULT AND DISCUSSION

The combination of AMLO and OLME is used in the treatment of hypertension. Hence it is necessary to develop method for simultaneous estimation of AMLO and OLME. Previously reported spectrophotometric methods were found to be less sensitive and selective for estimation of both drugs.

In present communication we have succeeded in development and validation of simple, accurate, precise, sensitive and selective absorbance ratio method and dual wavelength method for simultaneous estimation of both drugs from tablet formulation.

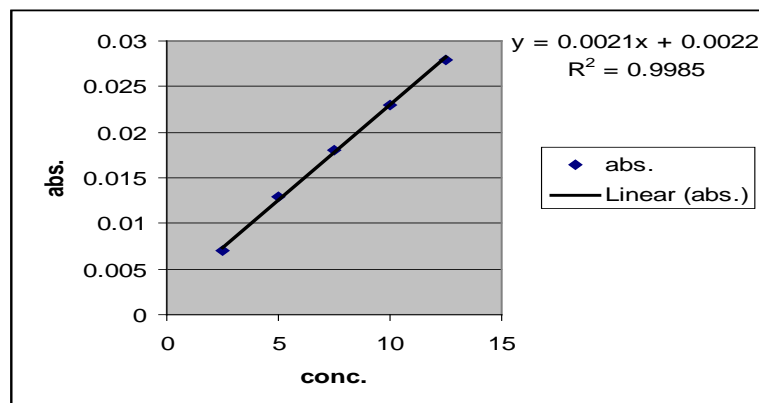


Fig. 5: Calibration curve at iso absorptive point 298.0 nm

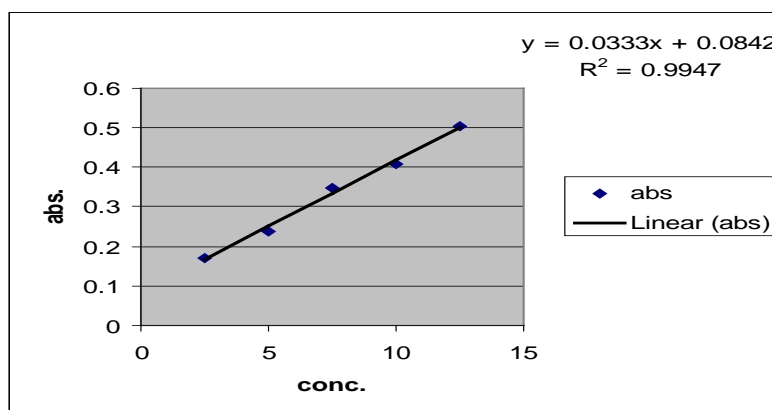


Fig. 6: Calibration curve of AMLO at 237.0 nm

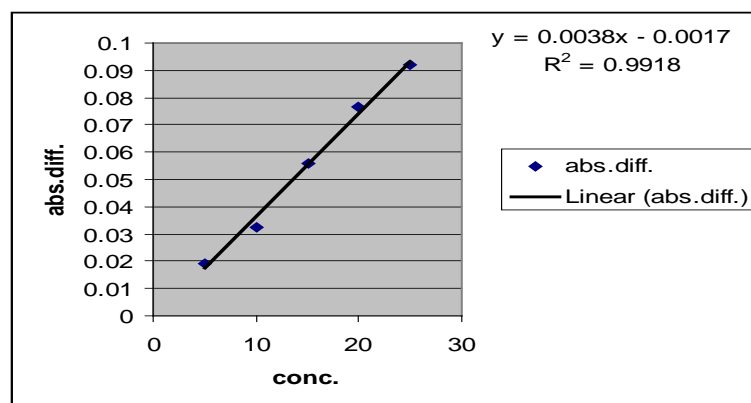


Fig. 7: Dual wavelength of AMLO at 232 & 241.6nm

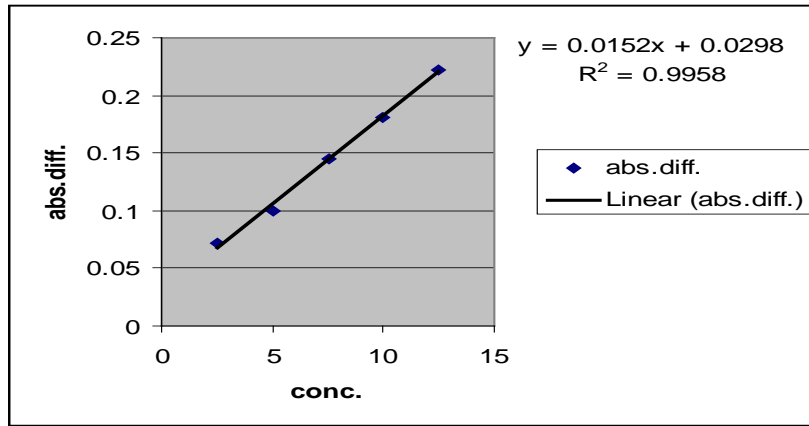


Fig. 8: Dual wavelength of OLME at 244 &255.4nm

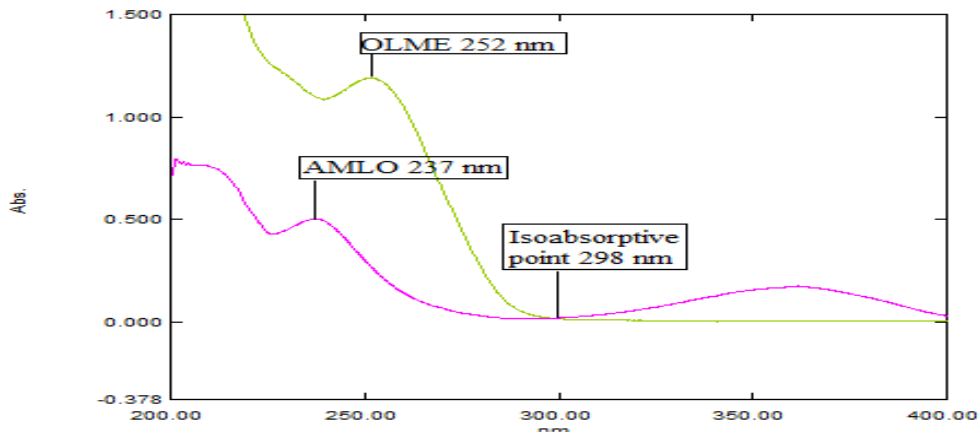


Fig. 9: Overlay absorption spectra of AMLO (12.5 µg/ml) and OLME (25 µg/ml)

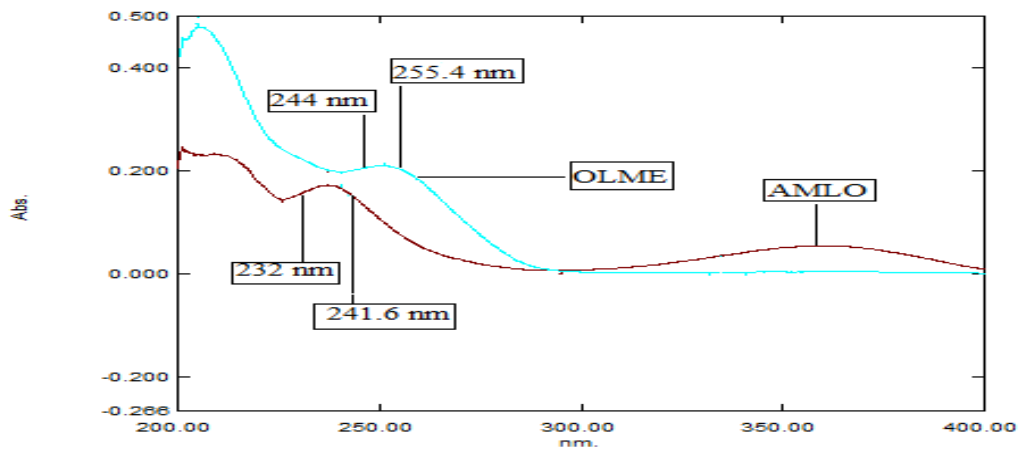


Fig. 10: Overlay absorption spectra of AMLO (232-241.6 nm) and OLME (244-255.4nm)

Table 1: Validation Parameters for Absorbance ratio method

Parameters	AMLO	OLME
Beer's law range	2.5-12.5 µg/ml	5-25 µg/ml
Wavelength (nm)	237	252
Correlation Coefficient	0.994	0.997
Slope	0.033	-0.013
Intercept	0.084	0.005
LOD (µg/ml)	0.03	0.03
LOQ (µg/ml)	0.09	0.09
% RSD		
Intraday precision	0.49-0.89	0.38-0.78
Interday precision	0.85-1.20	1.11-1.30

Table 2: Validation Parameters for Dual wavelength method

Parameters	AMLO	OLME
Beer's law range	2.5-12.5 µg/ml	5-25 µg/ml
Wavelength (nm)	232-241.6	244-255.4
Correlation Coefficient	0.9918	0.9958
Slope	0.0038	0.0152
Intercept	-0.0017	0.0298
LOD (µg/ml)	0.12	0.13
LOQ (µg/ml)	0.36	0.36
% RSD		
Intraday precision	0.36-0.72	0.68-0.96
Interday precision	1.09-1.34	1.12-1.29

LOD=limit of detection; LOQ=limit of quantification; (%RSD) = % relative standard deviation

Table 3: Results of simultaneous estimation of marketed formulation for Method A & B

Method	Label claim (mg)		Amount found (mg)*		% Label claim	
	AMLO	OLME	AMLO	OLME	AMLO	OLME
A	5	20	5.05	19.98	101.0	99.90
B	5	20	5.01	19.99	100.2	99.95

*Each value is a mean of six observations.

Table 4: Recovery studies of AMLO and OLME

Method	Level of recovery	Amount taken(µg/ml)		Amount added (µg/ml)		Total amount found (µg/ml)*		% recovery	
		AMLO	OLME	AMLO	OLME	AMLO	OLME	AMLO	OLME
A	0%	2.5	10	0	0	2.5	10	100.0	100.8
	50%	2.5	10	1.25	5	3.75	15	99.46	98.86
	100%	2.5	10	2.5	10.0	5	20	102.6	99.35
	150%	2.5	10	3.75	15.0	6.25	25	101.56	99.12
B	0%	2.5	10	0	0	2.56	10.1	101.4	101
	50%	2.5	10	1.25	5	3.76	15.02	100.2	100.1
	100%	2.5	10	2.5	10.0	5.01	20.01	100.2	100.05
	150%	2.5	10	3.75	15.0	6.23	25.03	99.68	100.12

*Each value is a mean of three observations.

CONCLUSION

These validated methods are new, rapid, accurate, precise, sensitive, reproducible and can be employed for routine analysis for simultaneous estimation of Amlodipine Besylate and Olmesartan Medoximil in combined dosage form.

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