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Simultaneous Estimation of Amlodipine Besylate and Olmesartan Medoxomil in Combined Tablet Dosage Form Using UV-Spectroscopy and Reverse Phase High-Performance Liquid Chromatography Method

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ABSTRACT

Simple, accurate and precise ultraviolet spectrophotometric and reversed-phase high-performance liquid chromatographic (RP-HPLC) methods for simultaneous estimation of amlodipine besylate (AMLO) and olmesartan medoxomil (OLME) in combined tablet dosage form have been developed and validated. Beer's law was obeyed in the concentration range of 5-70 and 5-60 µg/mL in methanol at 238 nm and 257 nm for AMLO and OLME, respectively for simultaneous equation method. The RP-HPLC method uses a Shimadzu LC 10 AT_{VP} system with Luna C_{18} column and acetonitrile: phosphate buffer (pH 4.0) (42:58 v/v) as the mobile phase. The detection was carried out using a diode array detector set at 238 nm. Linearity of chromatographic method was found in the concentration range of 2-40 and 8–160 µg/mL for AMLO and OLME respectively. The recoveries were in the range of 99.79 ± 0.161% and 100.12 ± 0.446% for AMLO, 99.85 ± 0.275% and 99.98 ± 0.404% for OLME in simultaneous equation method and HPLC methods respectively. Both methods may be used for routine analysis of the drugs in a pharmaceutical formulation. Results of analysis were validated statistically.

Keywords: absorptivity, robustness, simultaneous equation, validation
Abbreviations: **AMLO**, amlodipine besylate; **OLME**, olmesartan medoxomil; **RP-HPLC**, Reverse phase high performance liquid chromatography

INTRODUCTION

Amlodipine besylate (AMLO) is chemically known as 3-ethyl-5-methyl2-[(2aminoethoxy)methyl]-4-(2-chlorophenyl)-6methyl-1,4-dihydropyridine-3,5 dicarboxylate belongs to the class of calcium channel blocker and used as an antihypertensive agent (Anonymous 2001; Block *et al*. 2002). Olmesartan medoxomil (Anonymous 2001; Block *et al*. 2002) is chemically known as (5-methyl-2-oxo-2H-1,3dioxol-4-yl)methyl 4-(2-hydroxypropan-2-yl)2-propyl-1-({4-[2-(2H-1,2,3,4-tetrazol-5 yl) phenyl]phenyl}methyl)-1H imidazole-5-carboxylate. OLME is angiotensin antagonist and it acts by blocking the binding of angiotensin II to the AT1 receptors in vascular muscle; it is therefore independent of angiotensin II synthesis pathways, unlike acetylcholinesterase (ACE) inhibitors. By blocking binding rather than synthesis of angiotensin II, Olmesartan inhibits the negative regulatory feedback on renin secretion. As a result of this blockage, Olmesartan reduces vasoconstriction and the secretion of aldosterone. This lowers blood pressure by producing vasodilation, and decreasing peripheral resistance. The chemical structures of AMLO and OLME are shown in **Fig. 1**.

A literature survey revealed that several analytical methods have been reported for the determination of AMLO in biological fluids and in bulk as well as pharmaceutical formulations (Bahrami *et al*. 2004; Basavaiah *et al*. 2005; Zarghi *et al*. 2005; Dongre *et al*. 2007; Kakde *et al*. 2008) including high-performance liquid chromatography (HPLC), fluoroscence detection, titrimetric method, simple UV spectrophotometry and visble spectrophotometry. Similarly several analytical methods have been reported for the determination of OLME in biological fluids and in bulk as well as pharmaceutical formulations (Captain *et al*. 2007; Kawasaki *et al*. 2008; Najmal *et al*. 2008; Lisiane *et al*. 2009; Rote *et al*. 2009).

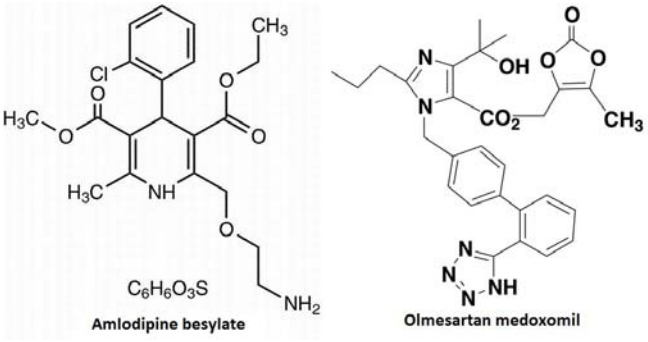

Fig. 1 Chemical structures of AMLO and OLME.

This paper describes simple, accurate, precise, and sensitive UV-spectrophotometric and reversed-phase (RP)-HPLC methods for simultaneous determination of AMLO and OLME in a combined tablet dosage form. The proposed methods were optimized and validated according to International Conference on Harmonization (ICH) guidelines (Anonymous 1996).

MATERIALS AND METHODS

Drugs and chemicals

Acetonitrile and Methanol were purchased from Merck (Mumbai, India). All other reagents used were of analytical grade for the

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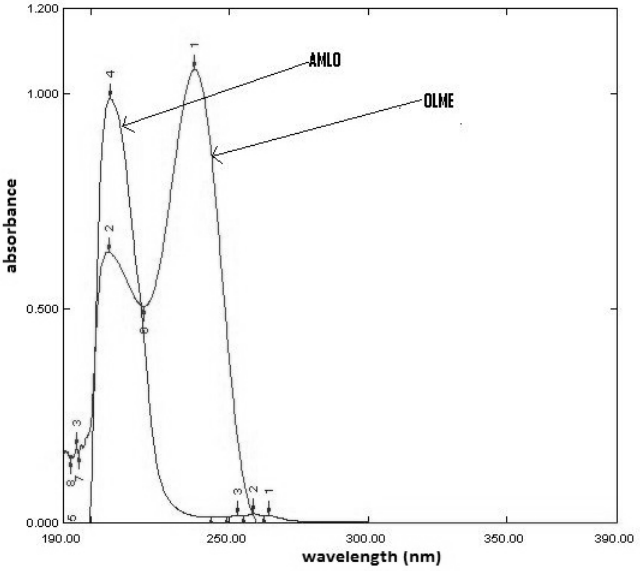

Fig. 2 Zero order overlain spectra of AMLO and OLME.

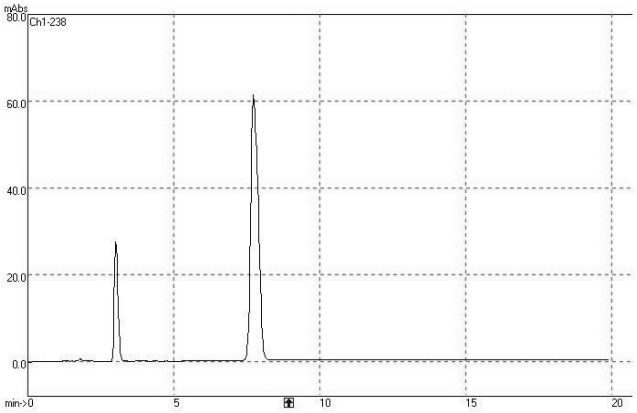

Fig. 3 Chromatogram of OLME and AMLO in tablet dosage form.

spectrophotometric determination and of HPLC grade for the HPLC method. Gratis samples of AMLO and OLME were provided by Cipla Pvt. Ltd., Vikroli (Mumbai, India). The pharmaceutical dosage form used in this study was OLMESAR A tablets labeled to contain 5 and 20 mg of AMLO and OLME, respectively (Macleods Pharmaceuticals ltd, India).

Instruments

A UV-Visible double beam spectrophotometer (Model 1601; Shimadzu, Japan) with 1cm matched quartz cells and UV probe software was used for the spectrophotometric method. A HPLC system consisting of LC 10 ATvp pump equipped with diode array detector (Shimadzu, Japan) and Luna C18 (4.6 mm id) column and class M10A software was used for chromatographic determination. A Rheodyne (Rohnert Park, CA) injector with 20 µL loop was used for injecting the sample.

Method I: Simultaneous equation method

A stock solution of each drug having a concentration of 1 mg/mL (i.e.1000 µg/mL) was prepared by dissolving AMLO and OLME separately in 100 mL methanol. Appropriate dilution were prepared using methanol from the stock solutions 1000 µg/mL of AMLO and OLME to get aliquots of the concentration of 5, 10, 15, 20, and 25 µg/mL. Zero order overlain spectrum is presented in **Fig. 2**. Determinations were carried out at 238 and 257 nm, the maximum absorbance wavelength (λ_{max}) of AMLO and OLME, respectively. The calibration curves were plotted from mean absorbance values of observation of the six replicate. The absorptivity values for both the drugs were determined at their respective λ_{max} by measuring absorbance values for working standards of AMLO and OLME.

Analysis of tablet formulation: Twenty tablets were weighed accurately, and a quantity of tablet powder equivalent to 5 mg AMLO and 20 mg OLME was transferred to a 100 mL volumetric flask, 70 mL methanol was added, and the flask was shaken vigorously for 5 min, and sonicated for 10 min. The volume was made up to the mark with methanol. The solution was filtered and further diluted with methanol to obtain a concentration within the Beer's law range. The absorbance of sample was measured at 238 and 257 nm. The contents of AMLO and OLME were calculated using linear regression analysis (Gandhi *et al*. 2008).

Method II: RP-HPLC method

Mobile phase consisting of acetonitrile: phosphate buffer (pH 4.0) (42: 58 v/v; filtered through a 0.2 µm membrane filter, degassed and sonicated) was used with a flow rate of 1.8 mL/min at 238 nm (Gopinath *et al*. 2007; Gandhi *et al*. 2008; Sabnis *et al*. 2008). (a) Standard stock solutions: Standard stock solutions having concentration of 100 µg/mL were prepared by dissolving drugs separately in the mobile phase. (b) Preparation of the calibration curves: Appropriate aliquots of 2, 4, 6, 8, 10 µg/mL and 8, 16, 24, 32, 40 µg/mL of AMLO and OLME were prepared using standard stock solution. Each solution was injected, and chromatogram was recorded. Mean retention times for AMLO and OLME were found to be 2.89 and 8.03 min, respectively. The peak area of AMLO and OLME were noted, and respective calibration curves were plotted as peak area against concentration of each drug.

(c) Procedure for analysis of tablet formulation: Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 5 mg AMLO and 20 mg OLME weighed and transferred to a 50 mL volumetric flask containing about 70 mL mobile phase, ultrasonicated for 10 min, and the volume was made up to the mark with the mobile phase. The solution was filtered through No. 14 Whatman paper , 0.2 mL filtrate was transferred to a 10 mL volumetric flask and the volume was made up to the mark with the mobile phase. The sample solution was injected, and the peak areas were recorded. A representative chromatogram is given in **Fig. 3**.

(d) Robustness studies: The influence of small, deliberate variations of the analytical parameters on the retention time of the drugs was examined. The following factors were selected: the wavelength at which the drugs were recorded (238 ± 1 nm) and the flow rate of the mobile phase (1.8 ± 0.02 mL/min). One factor at the time was changed to evaluate the effect. The solutions containing 10 µg/mL AMLO and 40 µg/mL OLME were injected in to the column. Five replicate analyses (n = 5) were conducted at 3 levels of the factor (–, 0, +).

(e) Recovery studies: To study the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample at 3 different levels: 80, 100, and 120% of the target concentration.

(f) Precision: Precision of the method was checked using 3 replicate readings at 3 concentration levels of within range expressed as RSD values.

Statistical analysis

Means, standard deviation (SD), relative standard deviation (RSD), and linear regression analyses were calculated using Microsoft Excel 2003.

RESULTS AND DISCUSSION

For spectroscopic method (Method I), methanol was used as common solvent. Linearity range of AMLO and OLME was found to be in the concentration range of 5-70 and 5-60 µg/mL having a correlation coefficient of 0.9987 and 0.9995 at 238 and 257 nm, respectively.

Chromatographic conditions (Method II) in RP-HPLC method were optimized to achieve the best resolution and peak shape for AMLO and OLME. Different mobile phases

Table 1 Regression analysis of calibration curves of method I and II.

Parameters	Method I		Method II	
	AMLO	OLME	AMLO	OLME
λ_{max}	238	257	238[a]	238[a]
Beer's law limit µg/mL	5-70	5-70	2-40	2-160
Correlation coefficient	0.9987	0.9995	0.9992	0.9989
Molar absorptivity	0.091	0.053	-	-
Linear regression equation[b]				
Intercept	0.0131	0.0203	1778.4	17765
Slope	0.0337	0.0416	24545	26612
SD[c]	0.0337	0.0416	2440.74	8087.52
Detection limit, µg/mL	0.54	0.44	0.32	1.002
Quantitation limit, µg/mL	1.66	1.34	0.99	3.039

[a] Detection wavelength for HPLC method.
[b] y = mx + c, where y is the absorbance and x is the concentration (µg/mL).
[c] SD = standard deviation.

Table 2 System suitability parameters for RP-HPLC method.

Parameters	AMLO	OLME
Calibration range, µg/mL	2-40	8-160
Theoretical plate number	3387	5826
HETP[a]	0.0073	0.0042
Tailing factor	1.21	1.05
Capacity factor (k')	0	1.78
Resolution	-	16.58

[a] HETP = Height equivalent to theoretical plate, cm

Table 3 Robustness data in terms of retention time for AMLO and OLME.

Level	Wavelength[b]		Flow rate[c]	
	AMLO	OLME	AMLO	OLME
-	2.89 ± 0.049	8.03 ± 0. 15	2.89 ± 0.072	8.03 ± 0.065
0	2.90 ± 0.066	8.04 ± 0.18	2.90 ± 0.092	8.04 ± 0.075
+	2.90 ± 0.110	8.04 ± 0. 14	2.90 ± 0.54	8.04 ± 0.069

[a] Mean ± SD, n = 6.
[b] 238 ± 1 nm.
[c] 1.8 ± 0.02 mL/min.

Table 4 Results of analysis of commercial formulation.

Method	Label claim, mg/tablet		% Claim, estimated[a]	
	AMLO	OLME	AMLO	OLME
I	5	20	99.6 ± 4.30	99.50 ± 1.16
II	5	20	100.03 ± 1.098	99.96 ± 0.517

[a] Average of 6 determinations.

containing acetonitrile and phosphate buffer (pH 4.0) were examined, and the mobile phase acetonitrile: phosphate buffer (pH 4.0) (42: 58 v/v) was found to be optimum at 238 nm, at which the best detector response for both the substances was obtained. Linear calibration curves were obtained for AMLO and OLME in the spectrophotometric and RP-HPLC methods. **Table 1** summarizes the Beer's law limit, linear regression equation, correlation coefficient, standard deviations (SD), limit of detection (LOD) and limit of quantitation (LOQ) values for both methods. System suitability parameters for the RP-HPLC method are listed in **Table 2**.

Robustness studies of the HPLC method, carried out after deliberate alterations of the analytical wavelength and flow rate of mobile phase, indicated that small changes of these operational parameters do not lead to significant variations. The effect of a single factor at two levels indicated that the selected factors remained unaffected by small variations of these parameters. Therefore, this method is suitable for in routine analysis (**Table 3**).

The proposed methods were also evaluated in the assay of commercially available tablets containing AMLO and OLME. Five replicate determinations were performed on the accurately weighed amounts of tablets. For AMLO, recovery (mean, %, ± SD, n = 3) was found to be 99.79 ± 0.161% and 100.12 ± 0.446% for Methods I and II, respectively. For OLME, recovery was found to be 99.85 ± 0.275% and 99.98 ± 0.404% for Methods I and II, respectively (**Tables 4, 5**).

CONCLUDING REMARKS

The proposed spectrophotometric and RP-HPLC methods were found to be simple, fast, accurate, precise, and sensitive. Thus, they may be used for routine analysis of AMLO and OLME in combined tablet dosage form.

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Table 5 Recovery studies of AMLO and OLME by Methods I and II.

Drug	Concentration taken, µg/ml for methods	Concentration added, µg/ml for methods	Total concentration found µg/ml		Recovery, %[a]	
			Method I	Method II	Method I	Method II
AMLO	5	4	8.964	8.897	99.61 ± 0.14	99.75 ± 0.06
	5	5	9.971	10.002	99.71 ± 0.20	100.02 ± 0.27
	5	6	11.005	11.066	100.05 ± 0.14	100.6 ± 1.00
OLME	20	16	35.967	35.895	99.91 ± 0.10	99.71 ± 0.18
	20	20	39.992	40.160	99.98 ± 0. 07	100.4 ± 0.66
	20	24	44.022	43.925	100.05 ± 0.14	99.83 ± 0.35

[a] Mean ± standard deviation (n=3)

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