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Validated HPTLC method for simultaneous quantitation of Olmesartan medoximal and Amlodipine besylate in bulk drug and formulation

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Abstract

A new, simple, precise, and accurate HPTLC method for simultaneous quantitation of olmesartan medoxomil (OLME) and amlodipine besylate (AMLO) as the bulk drug and in tablet dosage forms have been developed. Chromatographic separation of the drugs was performed on aluminum plates precoated with silica gel 60 F254 as the stationary phase and the solvent system consisted of Chloroform: Methanol: Toluene: Acetic acid (8:1:1:0.1 v/v/v/v). Densitometric evaluation of the separated zones was performed at 254 nm. The two drugs were satisfactorily resolved with R_F values of 0.45 ± 0.02 and 0.15 ± 0.02 for OLME and AMLO, respectively. The accuracy and reliability of the method was assessed by evaluation of linearity (800-5600ng spot^{-1} for OLME and 200-1400ng spot^{-1} for AMLO), precision (intra-day RSD 0.65–1.33% and inter-day RSD 0.97–1.45% for OLME, and intra-day RSD0.83–1.35% and inter-day RSD1.42–1.68% for AMLO), accuracy for OLME and AMLO afford 98-102%, and specificity, in accordance with ICH guidelines.

Key words: Olmesartan medoxomil, Amlodipine besylate, densitometry, validation, quantification.

INTRODUCTION

OLME chemically is 2,3-dihydroxy-2-butenyl-(1-hydroxy-1-methyl ethyl)-2-propyl-1-[P-(O-1H-tetrazole-5-ylphenyl)benzyl] imidazole-5- carboxylate, cyclic 2,3-carbonate. Olmesartan medoxomil is a prodrug, which, after ingestion, liberates the only active metabolite, olmesartan. Olmesartan is a competitive and selective AII type 1 receptor antagonist. The hydrolysis of OLMD occurs readily by the action of esterases which are present abundantly in the gastrointestinal tract, liver and plasma and is used alone or with other antihypertensive agents to treat hypertension [1-2]. Amlodipine besylate (AMLO) is chemically know as 3-ethyl-5-methyl (±)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1, 4-dihydro-6-methyl-3, 5-pyridine

dicarboxylate, monobenzene sulphonate is a long-acting calcium channel blocker [3-4]. Most hypertensive patients require more than one agent in order to achieve adequate blood pressure (BP) control. Fixed-dose combination antihypertensive treatments such as OLME/AMLO have advantages over mono therapy including increased efficacy, reduced side effects and lower costs. Literature survey shows that several HPLC methods are available for estimation of OLME and AMLO as an individual as well as in combination. HPLC methods for estimation of OLME in human plasma have been reported [5-8]. HPLC methods for estimation of OLME in tablet dosage forms have also been studied [9-10]. Reverse phase HPLC method for simultaneous determination of OLME and ramipril is also reported [11]. HPLC methods for estimation of AMLO in human plasma have been reported [12-17]. Literature reveals that, many HPLC methods for estimation of AMLO in tablet dosage forms have been investigated [18-20]. Reverse phase HPLC method for determination of OLME and ramipril has been studied [21-25]. HPTLC method for quantification of AMLO and valsartan is reported [26].HPTLC method for the estimation of AMLO and atenalol are also reported [27].Literature survey indicate that many methods are available for estimation of OLME and AMLO by HPLC, however HPTLC methods have not been investigated for the simultaneous determination of OLME and AMLO in combined dosage form. Hence it was decided to develop simple, precise and accurate HPTLC method for simultaneous determination of binary drug formulation. The proposed method was optimized and validated as per the ICH guidelines [28].

MATERIALS AND METHODS

Chemicals and Equipments

All chemicals and reagents of analytical grade were purchased from Merck Chemicals, Mumbai, India. Pure drug sample of AMLO, (% purity 99.8) was kindly supplied as a gift sample by Sanmour Pharmaceuticals Pvt. Ltd. Thane, India and pure drug sample of OLME (% purity 99.3) was gifted by Sun Pharmaceuticals Pvt. Ltd. Mumbai, India. Tablet used for analysis were OLMY-A (Batch No. OA006) manufactured by Burgeon Pharmaceuticals Pvt. Ltd. Chennai, India containing OLME 20mg and AMLO 5mg per tablet. HPTLC system used for analysis Camag HPTLC system. The samples were spotted in the form of bands with a Camag 100 µl sample (Hamilton, Bonaduz, Switzerland) syringe on precoated silica gel aluminum plate 60 F_{254} (20×10) with 250 µm thickness; (E MERCK, Darmstadt, Germany) using a Camag Linomat 5 sample applicator (Switzerland). The linear ascending development was carried out in 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) using mobile phase. TLC plates were dried in a current of warm air with the help of a hair drier. Densitometric scanning was performed on Camag TLC scanner 3 in the reflectance-absorbance mode at 254 nm for all measurements and operated by Camag WINCATS software version 1.4.4. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum 200 to 400.

Preparation of Standard Stock Solutions

50 mg of each drug OLME and AMLO were weighed separately and dissolved in 20 ml of methanol and then volume was made up to 50 ml so as to get the concentration 1 mg mL^{-1}. From each of these solutions 1ml of solution was pipette out and transferred to 10 ml volumetric flasks and volume was made up to the mark using methanol so as to get the concentration 100 µg mL^{-1}. The stock solution was stored at 2–8 ^{0}C protected from light.

Optimization of the HPTLC Method

Chromatographic separation studies were carried out on the stock solution of OLME and AMLO. Initially on the plates 10µl of stock solution was applied as band 8 mm of width. Plates were developed by linear ascending development using neat solvents like toluene, hexane, methanol,

chloroform, dichloromethane, ethyl acetate, acetone, acetonitrile, etc. without chamber saturation. Based on the results of these initial chromatograms binary and ternary mixtures of solvents were tried to achieve optimum resolution between AMLO and OLME respectively. After several trials, mixture of Chloroform: Methanol: Toluene: Acetic acid (8:1:1:0.1 v/v/v/v) was chosen as the mobile phase for analysis. Other chromatographic conditions like chamber saturation time, run length, sample application rate and volume, sample application positions, distance between tracks, detection wavelength, were optimized to give reproducible R_F values, better resolution, and symmetrical peak shape for the two drugs. Good resolution with R_F value of 0.15 for AMLO and 0.45 for OLME was obtained when densitometric scanning was performed at 254 nm (Fig.I). The spot appeared more compact and peak shape more symmetrical when the TLC plates were pretreated with methanol and activated at 110^oC for 5 min. Well-defined spots of standard along with its degradation products were obtained when the chamber saturation time was optimized at 20 min at room temperature.

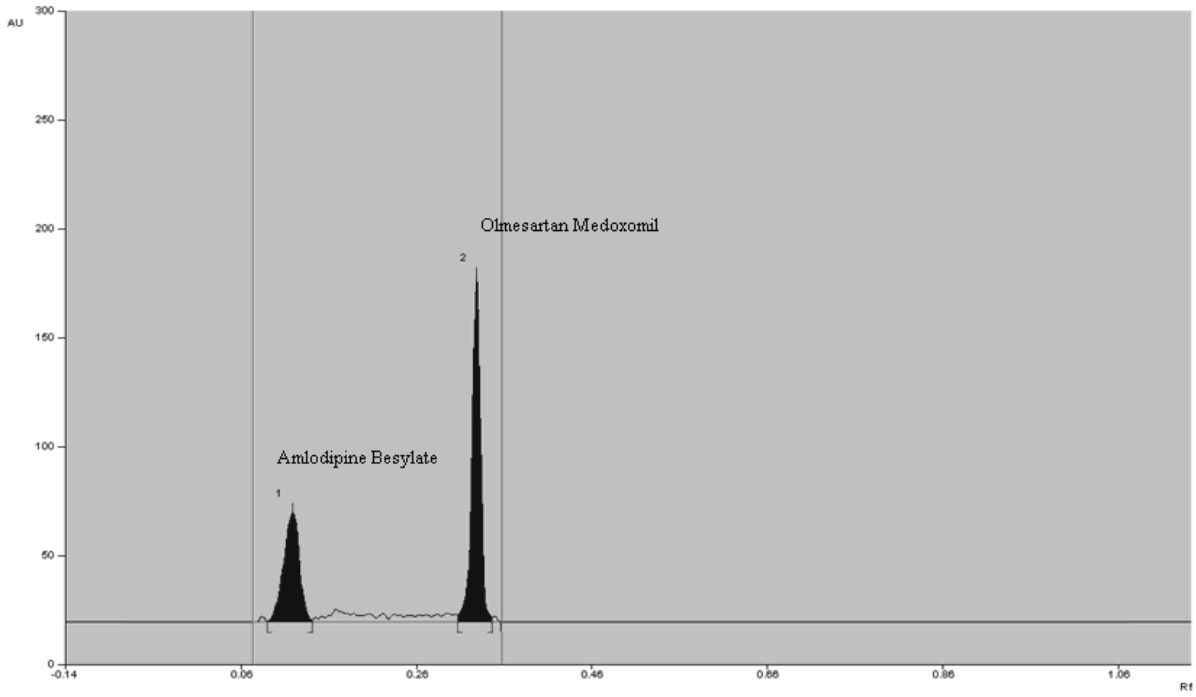

Fig.I Densitogram of Olmesartan Medoximal (R_f 0.45) and Amlodipine Besylate (R_f 0.15) of formulation showing no interference of excipients in analysis

Validation of the Method

Linearity and Range

Stock standard solution was prepared by dissolving 10 mg of OLME and AMLO in 10mL methanol (1000 µg mL^{-1}) separately. The standard solutions were prepared by dilution of the stock solution with methanol to reach a concentration 0.1 mg mL^{-1}. From each stock solution OLME and AMLO was separately spotted on the TLC plate to obtain final concentration 800-5600ng $spot^{-1}$and 200-1400ng $spot^{-1}$of OLME and AMLO respectively. Each concentration was spotted 3 times on the TLC plate. The plate was developed on previously described mobile phase. Peak areas were plotted against corresponding concentrations to obtain the calibration graphs.

Precision

Three sets of three different concentrations of standard solution of OLME (2400, 3200, 4000ng spot^{-1}) and AMLO (600, 800, 1000ng spot^{-1}) were prepared. The intra-day precision of the developed TLC method was determined by preparing the tablet samples of the same batch in nine determinations with three concentrations and three replicate each on same day. The inter-day precision was also determined by assaying the tablets in triplicate per day for consecutive 3 days.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD was calculated from the formula LOD = 3.3σ /S, where σ = Standard deviation of the response calibration curve, S = Slope of the calibration curve and LOQ was calculated from the formula LOQ = 10σ /S, where σ = Standard deviation of the response calibration curve, S = Slope of the calibration curve.

Robustness of the Method

By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different composition like Chloroform: Methanol: Toluene: Acetic acid (8.1: 1:1:0.1 v/v/v/v), (7.9: 1.2:1:0.1 v/v/v/v), (8: 1:1.2:0.1 v/v/v/v), (8: 1:1:0.2 v/v/v/v) were tried and chromatograms were run. The plates were prewashed by methanol and activated at 110^{o}C for 5, 10, 15 min respectively prior to chromatography. Time from spotting to chromatography and from chromatography to scanning was varied from 0, 20, 40 and 60 minutes. In this also detection wavelength(+/-1nm) is alerted ,duration of saturation(+/-5min), development distance (+/-1cm) changes Robustness of the method was done at three different concentration levels 200, 400, 600ng spot^{-1}and 800, 1600, 2400ng spot^{-1} for AMLO and OLME, respectively.

Specificity

The specificity of the method was determined by analyzing standard drug and test samples. The spot for AMLO and OLME in the samples was confirmed by comparing the RF and spectrum of the spot to that of a standard. The peak purity of AMLO and OLME was determined by comparing the spectrum at three different regions of the spot i.e. peak start (S), peak apex (M) and peak end (E).

Accuracy

Accuracy of the method was carried out by applying the method to drug sample(OLME and AMLO combination tablets) to which known amounts of OLME and AMLO standard powder corresponding to50, 100 and 150% of label claim had been added (standard addition method), mixed and the powder was extracted and analyzed by running chromatograms in optimized mobile phase.

Analysis of a Marketed Formulation

Quantity of tablet powder equivalent to 20 mg of OLME and 5 mg of AMLO was weighed and transferred to a 100 ml volumetric flask containing about 70 ml of mobile phase, ultrasonicated for 5 min, filtered, filter was washed with methanol, washing transfers to flask and volume was made up to the mark with the mobile phase to get sample stock solution. The sample stock solution was suitably diluted to get solutions of concentrations of 64µgmL^{-1} of OLME (16 µgmL^{-1} AMLO). These solutions were spotted keeping appropriate distance between spots.

RESULTS AND DISCUSSION

HPTLC methods are significant methods for Quality assurance of drug molecules. HPTLC has emerged as a routine analytical technique due to its advantages of low operating costs, high sample throughput and the need for minimum sample preparation. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike LC thus reducing the analysis time and cost per analysis. Hence, the method was developed for OLME and AMLO as bulk drug and in pharmaceutical formulation. The method was validated and found to be suitable for routine analysis of the selected drugs.
The results of validation studies on simultaneous estimation method developed for OLME and AMLO in the current study involving Chloroform: Methanol: Toluene: Acetic acid (8:1:1:0.1 v/v/v/v) as the mobile phase for TLC are discussed below.

Linearity

The drug response was linear (*r*2 = 0.9991 for OLME and 0.9999 for AMLO) over the concentration range between 800–5600ng spot^{-1} for OLME and 200–1400ng spot^{-1} for AMLO.

Precision

The results of the repeatability and intermediate precision experiments are shown in Table I. The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were <2%, respectively as recommended by ICH guidelines.

Table I Results of Precision

Drug	Precision of the Method [b] (n=6)		
	Actual Conc. (µgmL^{-1})	Measured conc. (µgmL^{-1}), % R.S.D	
		Repeatability	Intermediate precision
OLME	2400	2400.07, 0.65	2421,0.97
	3200	3211,1.33	3221,1.45
	4000	4025,1.28	4111,1.37
AMLO	600	623.01,0.83	601.03,1.68
	800	811,1.35	816.12,1.42
	1000	1012.01,1.22	1121.23,1.45

LOD and LOQ

The LOD and LOQ were found to be 200ngspot^{-1} and 400ngspot^{-1} respectively for OLME and 80ngspot^{-1} and 150ngspot^{-1} respectively for AMLO.

Table II Robustness Testing (n = 6)

Parameter	SD of peak area		% RSD	
	OLME	AMLO	OLME	AMLO
Mobile phase composition	1.75	0.60	1.26	0.84
Amount of mobile phase	1.64	1.70	1.32	1.23
Time from spotting to chromatography	0.83	0.71	0.60	0.90
Time from chromatography to scanning	0.60	1.23	0.40	0.36
Plate pretreatment	0.83	1.22	0.62	0.84
Measurement wavelength(nm)	1.74	1.84	1.25	1.31
Development distance	1.68	1.36	1.51	1.45
Saturation time	1.36	0.93	1.16	1.12

Robustness of the Method

The standard deviation of the peak areas was calculated for each parameter and the % RSD was found to be less than 2%. The low values of the% RSD, as shown in Table II indicated robustness of the method.

Specificity

The peak purity of both drugs was assessed by comparing the respective spectra of standard drugs and samples at peak start, peak apex and peak end positions of the spot i.e., *r* (S, M) = 0.9988 and *r* (M, E) = 0.9980 for OLME and r (S, M) = 0.9995 and *r* (M, E) = 0.9992 for AMLO.

Recovery Studies

Chromatogram was developed and the peak areas were noted. At each levels of the amount, three determinations were performed. As shown from the data in Table III good recoveries of the OLME and AMLO in the range from 99.7 to 100.6% were obtained at various added concentrations.

Table III: Recovery Studies (n = 6)

Drug	Label claim (mg per tablet)	Amount Added (%)	Total amount (ng $spot^{-1}$)	Amount recovered (ng) ± %RSD	Recovery (%)
OLME	20	50	800	801.3,0.63	100.17
		100	1600	1598.14,0.59	99.89
		150	2400	2410.23,0.94	100.42
AMLO	5	50	200	201.32,,0.67	100.66
		100	400	401.21,0.51	100.31
		150	600	598.47,1.03	99.74

Analysis of a Formulation

Experimental results of the amount of OLME and AMLO in tablets, expressed as a percentage of label claims were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients which are normally present in tablets (Fig. I). The drug content was found to be 98.68% ± 0.73.Two different lots of OLME and AMLO combination tablets were analyzed using the proposed procedures.

CONCLUSION

A simple, rapid, less expensive, accurate, reliable HPTLC method has been developed for analysis of OLME and AMLO as bulk drug and in pharmaceutical formulation without any interference from excipients. The data shows that the method is suitable for routine analysis of the drugs. The method can be used to study the degradation kinetics of OLME and AMLO and also for its estimation in plasma and other biological fluids.

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