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Development and validation of a RP-HPLC method for simultaneous estimation of Olmesartan Medoxomil and Amlodipine Besylate in tablet dosage form

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ABSTRACT

A simple, precise, rapid and accurate RP HPLC method has been developed for the simultaneous estimation of olmesartan medoxomil and amlodipine besylate in tablet formulations. The chromatographic separation was achieved on a Waters Symmetry C_{18} column (250 mm x 4.6 mm, 5.0 µ particle size) using Acetonitrile: Methanol: water (60: 28: 12 v/v/v) mobile phase. Ortho-phosphoric acid was used to adjust pH to 3.2, flow rate was 0.6 ml/min and column was maintained at 30^0C. Quantification and linearity was achieved at 254 nm over the concentration range of 2 to 128µg /ml for olmesartan medoxomil and 0.5 to 32µg/ml for amlodipine besylate. The method was validated for specificity, linearity, accuracy, precision, LOD, LOQ and robustness.

Key words: Amlodipine besylate, Olmesartan medoxomil**,** RP HPLC, Validation, Formulation.

INTRODUCTION

A simple, precise, rapid and accurate RP HPLC method has been developed for the simultaneous estimation of olmesartan medoxomil and amlodipine besylate in tablet formulations. The chromatographic separation was achieved on a Waters Symmetry C_{18} column (250 mm x 4.6 mm, 5.0 µ particle size) using Acetonitrile: Methanol: water (60: 28: 12 v/v/v) mobile phase. Ortho-phosphoric acid was used to adjust pH to 3.2, flow rate was 0.6 ml/min and column was maintained at 30^0C. Quantification and linearity was achieved at 254 nm over the concentration range of 2 to 128µg /ml for olmesartan medoxomil and 0.5 to 32µg/ml for amlodipine besylate. The method was validated for specificity, linearity, accuracy, precision, LOD, LOQ and robustness.

Olmesartan medoxomil (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 OLME 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 known 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. 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 [5-7]. HPLC methods for estimation of OLME in tablet dosage forms have also been reported [8-10]. RP- HPLC method for determination of OLME and ramipril is also reported [9]. HPLC methods for estimation of AMLO in human plasma have been reported [10-15]. HPLC methods for estimation of AMLO in tablet dosage forms have also been reported [16-18]. Several HPLC methods for AMLO combination are reported [19-23]. HPLC method for the estimation of AMLO and atenalol are also reported [24]. Literature survey also revealed that no method is available for simultaneous determination of OLME and AMLO in combined dosage form by reversed-phase liquid chromatographic method. Aim of the present work was to develop simple, precise and accurate RP HPLC method for simultaneous determination of binary drug formulation. The proposed method was optimized and validated as per the ICH (International Conference on Harmonization) guidelines [25].

MATERIALS AND METHODS

Chemicals and Equipments

HPLC grade Acetonitrile and Methanol was purchased from Merck (Mumbai, India). HPLC grade ortho-phosphoric acid was purchased from Research lab fine chem. Industries (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. Pundhucherry, India containing OLME 20mg and AMLO 5mg per tablet. Waters HPLC system, Milford USA consisted of binary Pump (Waters 515), with Auto sampler (model Waters; 717) having injection capacity of 5-200 µl. Photo diode array (PDA) detector (Waters 2998) was used. Data was integrated using Waters Empower 2 system. A chromatographic separation was achieved on Waters Symmetry C_{18} column (250 mm x 4.6 mm, 5.0 µ particle size) and Kromasil C_{18} (250 mm × 4.6 mm, 5.0 µ) analytical column. The column was supported with waters symmetry C- 18, (3.9x20mm, 5.0 µ) guard column.

Standard solutions and calibrations graphs

The stock solution of OLME and AMLO was prepared by dissolving in methanol to obtain a final concentration of 1.0mg/ml. From this stock solution, Standards within a 2-128µg/ml and

0.5-32 µg/ml concentration range were prepared for OLME and AMLO respectively. A graph was plotted as concentration of drugs versus peak area response. It was found to be linear for both the analytes. From the standard stock solution, a mixed standard solution was prepared containing 64 µg/ml of OLME and 16 µg/ml of AMLO. The system suitability test was performed from six replicate injections of mixed standard solution.

Sample preparation

Quantity of tablet powder from 2o tablets equivalent to 20 mg of OLME (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 and volume was made up to the mark with the mobile phase and suitably diluted to get solutions of concentrations of 64 µg/ml of OLME (16 µg/ml AMLO). The sample solution was then filtered using 0.45 µ nylon filter and 20 micro liters of the test solution was injected and amounts of the drugs were calculated from chromatogram.

Method validation

Assay method precision was determined using nine-independent test solutions. The intermediate precision of the assay method was also evaluated using different analyst on three different days. The accuracy of the assay method was evaluated with the recovery of the standards from excipients. Three different quantities (low, medium and high) of the authentic standards were added to the placebo. The mixtures were extracted and were analyzed using the developed HPLC method. The LOD and LOQ for analytes were estimated by injecting a series of dilute solutions with known concentration. To determine the robustness of the method, the final experimental conditions were purposely altered and the results were examined.

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions

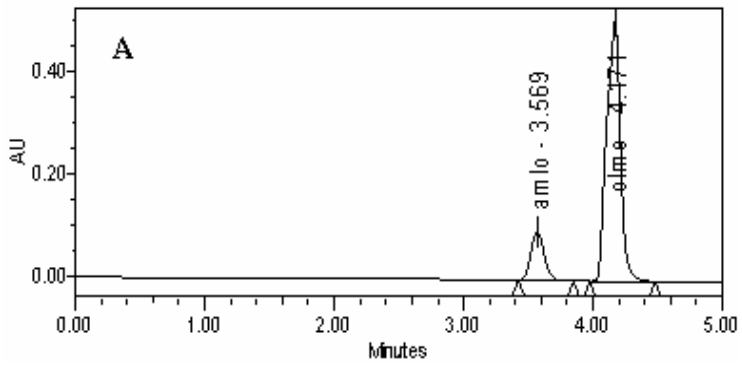
The HPLC method was optimized with a view to develop a reversed phase HPLC method for simultaneous estimation of OLME and AMLO in tablet dosage form. Pure drug was injected in different mobile phase compositions. Initially methanol and water in different ratios were tried. But in that, both drugs did not showed development. Hence, methanol was replaced by acetonitrile and different ratios were tried. In this mobile phase OLME and AMLO showed the development but resolution was not satisfactory. To achieve proper resolution mixtures of water, methanol and acetonitrile were tried, ultimately mobile phase with acetonitrile: methanol: water (60: 28: 12v/v/v), showed satisfactory development. To improve further peak sharpness pH selected was 3.2 and column maintained at 30^0 C and flow rate was adjusted to 0.6 ml/min. Now, mobile phase acetonitrile: methanol: water (60: 28: 12v/v/v), pH 3.2 adjusted with ortho-phosphoric acid and column temperature 30^0 C shown good resolution, peak shape and desired elution time. UV detection was carried out at 254 nm. Chromatogram showed symmetrical peaks with good shapes; tailing factor for OLME and AMLO was within range and the resolution of the standard drugs was satisfactory. Retention time of OLME was 4.1 min and that of AMLO was 3.5 min. The system suitability parameters observed by using this mobile phase were reported in Table I. The mobile phase and sample solutions was filtered using 0.45 µm membrane filter and was degassed by ultrasonication for 10 min prior to use.

Validation of Method

Specificity

The specificity of the HPLC method is illustrated in Fig. I where complete separation of OLME and AMLO was noticed in presence of tablet excipients. In addition there was no any interference at the retention time of OLME and AMLO in the chromatogram of placebo solution. In peak purity analysis with PDA, purity angle was always less than purity threshold for both the analytes. This shows that the peaks of analytes were pure and excipients in the formulation does not interfere the analytes.

Fig. I: A typical chromatogram of a tablet sample solution containing of 32 µg/ml of OLME and AMLO 8µg/ml



Accuracy

Accuracy of the method was calculated by recovery studies at three levels by standard addition method (Table I). The mean percentage recoveries obtained for OLME and AMLO were 99.65% and 101.25%, respectively.

Table I: System suitability parameters, results of precision and Accuracy

Compound	System Suitability		Precision of the Method [b] (n=9)			Recovery Study (n=3)	
	Parameter	Value	Actual Conc. (µg/mL)	Measured conc. (µg/ml), % R.S.D		Level	% Recovery, % R.S.D.
				Intra-day	Inter-day		
OLME	Resolution [a]	3.7492	8	32.15,0.12	32.3,0.11	80	98.90,0.21
	Theoretical plates [a]	6666	12	47.98,0.11	48.49,0.17	100	100.62,0.18
	Peak symmetry[a]	1.0962	16	64.55,0.08	63.71,0.28	120	99.42,0.22
	% R.S.D.	0.29					
AMLO	Resolution [a]	-	32	7.87,0.23	8.11,0.39	80	101.56,0.16
	Theoretical plates [a]	5829	48	12.36,0.20	12.09,0.23	100	100.74,0.36
	Peak symmetry[a]	1.1084	64	15.81,0.12	16.31,0.19	120	101.45,0.28
	% R.S.D.	0.34					

[a] USP-NF 29 section 621, pp. 2135. [b] Data expressed as mean for "measured concentration" values.

Precision

The intra- and inter-day variability or precision data are summarized in Table I. The intra-day precision of the developed LC method was determined by preparing the tablet samples of the same batch in nine determinations with three concentrations and three replicate each. The R.S.D.

of the assay results, expressed as a percentage of the label claim, was used to evaluate the method precision. The inter-day precision was also determined by assaying the tablets in triplicate per day for consecutive 3 days. The results indicated the good precision of the developed method (Table I).

Linearity

Linearity was determined for OLME in the range of 2-128µg/ml; and for AMLO, 0.5-32µg/ml. The correlation coefficient ('r') values for both the drugs were >0.999. Typically, the regression equation for the calibration curve was found to be y=41306x−1018 for OLME and y = 39140x−2061 for AMLO.

LOD and LOQ

LOD and LOQ of OLME and AMLO were determined by calibration curve method. Solutions of both OLME and AMLO were prepared in the range of 0.4-12 and 0.1-3µg/ml respectively and injected in triplicate. Average peak area of three analyses was plotted against concentration. LOD and LOQ were calculated by using following equations. LOD = (3.3 ×Syx)/b, LOQ= (10.0 ×Syx)/b

Where Syx is residual variance due to regression; b is slope. LOD and LOQ for OLME were 0.13 and 0.4 µg/ml respectively and for AMLO were 0.10 and 0.3 µg/ml, respectively.

Robustness

The mixed standard solution is injected in five replicates and sample solution of 100% concentration is prepared and injected in triplicate for every condition and % R.S.D. of assay was calculated for each condition. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust (Table II).A simple, specific, linear, precise, rapid and accurate RP HPLC method has been developed and validated for quantitative determination of OLME and AMLO in new tablet formulation. The method is very simple and specific as both peaks are well separated and there is no interference form excipient with total runtime of 5 min, which makes it especially suitable for routine quality control analysis work.

Table II: Results of robustness study

		Mean % assay(n=3), % R.S.D. of results	
Factor	Level	OLME	AMLO
P^H of mobile phase	3.1	99.50,0.18	99.50,0.22
	2.9	100.47,0.15	98.12,0.18
Flow rate (ml/min)	0.5	100.10,0.16	100.75,0.35
	0.7	99.66,0.33	101.25,0.34
Column oven temperature(◦C)	25	100.54,0.15	98.75,0.36
	35	100.88,0.54	101.00,0.38
% of ACN	34	100.07,0.17	101.63,0.17
	38	99.35,0.11	99.63,0.11
Measurement wavelength (nm)	253	99.27,0.51	99.8,1.17
	255	99.30,0.58	99.9,0.81
Separation Column	Column I[a]	98.63,0.11	101.75,0.21
	Column II[b]	100.35,0.18	100.36,0.51

[a]Symmetry C – 18, [b]Kromasil C – 18 column.

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