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METHOD DEVELOPMENT AND VALIDATION OF RP-UPLC METHOD FOR THE ESTIMATION OF AMLODIPINE AND OLMESARTAN MEDOXOMIL IN TABLET FORMULATION

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ABSTRACT

A new simple, accurate, precise and reproducible gradient phase ultra-performance liquid chromatography method was developed and fully validated for the estimation of Amlodipine Besylate and Olmesartan Medoxomil in pharmaceutical tablet dosage form gradientlly using acetonitrile: triethylamine buffer(pH4.0+0.5) as mobile phase and Acquity BEH C8 column (4.6x 150 mm,2.6µg) as stationary phase and chromatogram was recorded at 237nm at a flow rate of 0.4ml/min. The retention time of AML were 1.5to 2.8 and OLM 3.2 to 5.5 min respectively and showed a good linearity in the concentration range of 5-25µg/ml with a concentration coefficient (R) of 0.99956 and 0.99985 respectively. The developed UPLC method was validated with respect to specificity, linearity, precision, accuracy, ruggedness (reproducibility), robustness and stability. The recovery data was in the range of 98.0%to 102.0%. the proposed method was validated as per ICH guidelines and successfully as per ICH guidelines and successfully applied to the development and validation of AML and OLM in tablet formulation.

KEYWORDS: Amlodipine Besylate, Olmesartan Medoxomil, UPLC, new method development, validation.

INTRODUCTION

A simple, precise, rapid and accurate RP-UPLC method has been developed for the validation of olmesaratn medoxomil and amlodipine besylate in tablet formulations. The chromatographic separation was achieved on a Waters Symmetry C18 column (Acquity BEH C18, (50 x 2.1mm), 1.7µm) using Acetonitrile: Methanol: water mobile phase. Ortho phosphoric acid was used to adjust pH to 4.2, flow rate was 0.4 mL per minute.

Quantification and linearity were achieved at 254 nm over the concentration range of 20 µg per ml to 60 µg per ml of Amlodipine besylate and 40 µg per ml to 480 µg per ml of Olmesartan Medoxomil. The method was validated for specificity, linearity, accuracy, precision, LOD, LOQ and robustness. Olmesartan medoxomil is a prodrug that hydrolysed to olmesartan during absorption. It is an angiotensin II receptor antagonist used for hypertension and is chemically designated as 5-methyl-2-oxo-1,3-dioxolen-4-y1) methoxy-4-(1-hydroxy-1-methylethyl)-2-propyl-l-{4-[2-(tetrazol-5-y1)-phenyl]phenyl}methylimidazol-5 carboxylate. The Olmesartan Medoxomil drug is mainly used for hypertension in countries like Japan and US. The Medoxomil moiety, which is enclosed with this drug, has endogenous ester moiety responsible for releasing metabolites in the body. Olmesartan Medoxomil has a favorable safety and efficacy profile, with blood pressure-lowering effects comparable to those of other angiotensin receptor blockers (i.e., Losartan, Valsartan, Irbesartan).[2] Amlodipine besylate chemically designated as 2-[(2-aminoethoxy)-methyl]-4-(2-chlorophenyl) 1,4-dihydro-6-methyl-3,5-pyridine-dicarboxylic acid-3 ethyl-5 methyl ester, is a calcium channel blocker used to treat hypertension and angina. Amlodipine blocks the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscles. It is metabolized in the liver and the metabolites are mostly excreted in urine together with less than 10 % of a dose as unchanged drug. The pure Active Pharmaceutical Ingredient (API), used in this project, is manufactured by GLENMARK Company.The developed method has been validated by following several parameters as mentioned in ICH guideline.[13-15] i.e., linearity, specificity, accuracy, precision, robustness, ruggedness, stability.

MATERIAL AND METHOD

Chemicals and Equipment's

UPLC grade Acetonitrile and Methanol was purchased from Rankem Ltd, Mumbai. UPLC grade ortho phosphoric acid was purchased from from Rankem Ltd, Mumbai. Pure drug sample of AMLO and OLME was kindly supplied as a gift sample by R&D Laboratory,

Glenmark Research Centre, Mahape , Navi Mumbai. Tablet used for analysis were OLMY-A (Batch No. GPLA003) manufactured by Glenmark pharmaceutical, India containing OLME 40mg and AMLO 5mg per tablet. Waters UPLC 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 Acquity BEH C18, (50 x 2.1mm,1.7µm particle size).

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 80µg-320 µg/ml And 20 µg per ml to 60 µg per 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 11 µg/ml of AMLO. The system suitability test was performed from six replicate injections of mixed standard solution.

Sample preparation

Transfer 10 tablets into 250 ml volumetric flask. Add 25mL of water for complete dispersion of tablet then add 180 ml of diluent, sonicate for 15 minutes with intermittent vigorous shaking. Cool to room temperature and make up the volume with diluent. Centrifuge the solution for 10 min. at 4000 rpm. Further dilute 5 ml supernatant solution to 25 ml with diluent. Filter this solution through 0.22µ Nylon syringe filter.

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 analysed using the developed UPLC 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 UPLC method was optimized with a view to develop a reversed phase UPLC 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 300 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 orthophosphoric acid and column temperature 300 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.

Validation of Method

Specificity

The specificity of the UPLC 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.

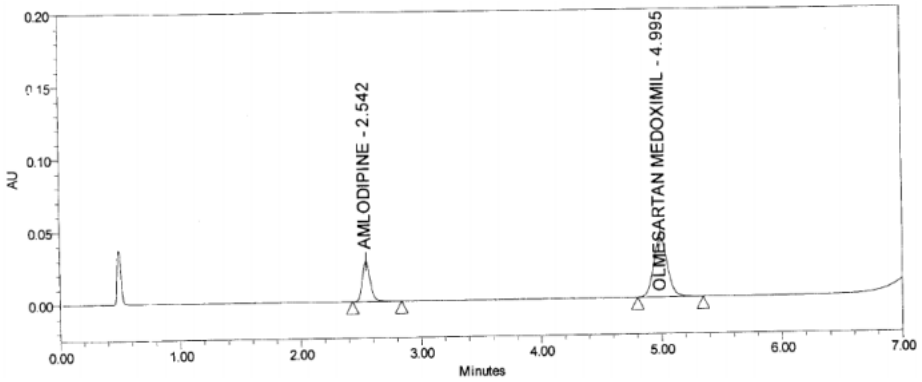

Fig. A typical chromatogram of a tablet sample solution.

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 1: System suitability parameters, results of precision and accuracy.

Compound	System Suitability		Precision of the method (n=9)			Recovery study(n=3)	
	Parameter	Value	Actual conc. (µg/ml)	Measured conc. (µg/ml) %RSD		Level	% recovery %RSD
				Intra-day	Inter-day		
OLME	Resolution	3.5359	8	29.14,0.10	29.5,0.9	80	98.85,0.20
	Theoretical plates	39002	12	40.25,0.09	40.46,0.15	100	100.58,0.17
	Peak symmetry	1.0736	16	60.51,0.03	59.45,0.21	120	99.40,0.19
	%RSD	0.29					
AMLO	Resolution	-	32	8.52,0.20	9.10,0.21	80	101.50,0.35
	Theoretical plates	41432	48	12.64,0.21	12.59,0.20	100	100.62,0.40
	Peak symmetry	1.1056	64	15.58,0.19	15.32,0.19	120	101.58,0.25
	%RSD	0.27					

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 1).

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=26278x-4960 for OLME and y = 32160x-1680 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 UPLC 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 2: Results of robustness study.

		Mean %assay(n=3), %R S D of results	
Factor	**level**	**OLME**	**AMLO**
pH of mobile phase	2.9	100.47,0.13	100.47,0.16
	2.5	101.10,0.15	98.23,0.15
Flow rate	0.5	100.10,0.15	100.50,0.30
	0.7	99.66,0.22	101.25,0.41
Column oven temperature(°C)	25	100.01,0.15	98.36,0.23
	35	100.52,0.12	100.23,0.25
% of ACN	34	100.07,0.12	101.96,0.24
	38	98.23,0.12	99.62,0.11
Measurement wavelength (nm)	253	99.24,0.52	99.8,1.15
	254	99.20,0.32	99.9,0.81
Separation column	Column I[a]	98.63,0.11	101.56,0.28
	Column II[b]	100.10,0.14	100.36,0.25

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