WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 15, 1252-1260. Research Article ISSN 2277– 7105

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF OLMESARTAN MEDOXOMIL AND AMLODIPINE BESYLATE IN PHARMACEUTICAL DOSAGEFORM

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Article Received on
20 October 2020,

Revised on 09 Nov. 2020,
Accepted on 29 Nov. 2020

DOI: 10.20959/wjpr202015-19329

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ABSTRACT

A RP-HPLC method was developed and validated for simultaneous determination of Olmesartan Medoxomil and amlodipine besylate in pharmaceutical dosage form. Separation was achieved on hypersil C18 (150mm X 4.6mm X 5µ) using a mobile phase consisting of0.1% Orthophosphoric acid: Methanol (60:40) at a flow rate of 1mL/min and UV detection at 254nm. Linearity was observed over the concentration range of 50 to 150 µg/ ml for bothr olmesartan medoxomil and amlodipine besylate. The average percentage recovery of the method was 101% for olmesartan medoxomil and 98% for amlodipine besylate. The method was validated as per ICH guidelines.

KEYWORDS: Olmesartan Medoxomil, Amlodipine Besylate, ICH, RP-HPLC, Validation.

INTRODUCTION

1) OLMESARTAN MEDOXOMIL

Structure

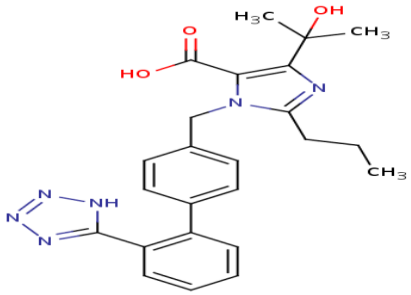

Fig no: 1 Structure of Olmesartan medoxomil.

Chemical names: 2, 3-Dihydroxy-2-butenyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-[p-(o-1H-tetrazol-5-ylphenyl) benzyl] imidazole-5-carboxylate, cyclic 2, 3-carbonate

IUPAC Name: 4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(1H-1, 2, 3, 4-tetrazol-5-yl) phenyl] phenyl} methyl)-1H-imidazole-5-carboxylic acid.

Molecular formula: $C_{24}H_{26}N_6O_3$

Molecular Weight: 558.59 gm/mol

Mechanism of action: Olmesartan is an ARB that selectively inhibits the binding of angiotensin II to AT1, which is found in many tissues such as vascular smooth muscle and the adrenal glands. This effectively inhibits the AT1-mediated vasoconstrictive and aldosterone-secreting effects of angiotensin II and results in a decrease in vascular resistance and blood pressure. Olmesartan is selective for AT1 and has a 12,500 times greater affinity for AT1 than the AT2 receptor. Also unlike the well-known ARB losartan, olmesartan does not have an active metabolite or possess uricosuric effects.

2) AMLODIPINE BESYLATE

Structure

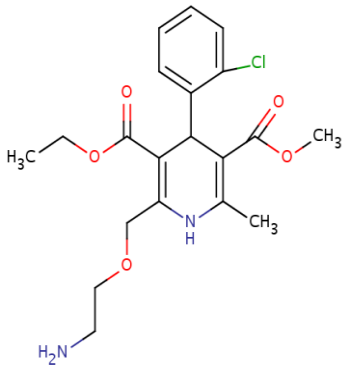

Fig no: 2 Stuctue ofAmlodipinebesylate.

IUPAC Name 3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate

Molecular formula: $C_{20}H_{25}ClN_2O_5$

Molecular Weight: 408.876gm/mol

Mechanism of action: Amlodipine decreases arterial smooth muscle contractility and subsequent vasoconstriction by inhibiting the influx of calcium ions through L-type calcium channels. Calcium ions entering the cell through these channels bind to calmodulin. Calcium-bound calmodulin then binds to and activates myosin light chain kinase (MLCK). Activated MLCK catalyzes the phosphorylation of the regulatory light chain subunit of myosin, a key step in muscle contraction. Signal amplification is achieved by calcium-induced calcium release from the sarcoplasmic reticulum through ryanodine receptors. Inhibition of the initial influx of calcium decreases the contractile activity of arterial smooth muscle cells and results in vasodilation. The vasodilatory effects of amlodipine result in an overall decrease in blood pressure. Amlodipine is a long-acting CCB that may be used to treat mild to moderate essential hypertension and exertion-related angina (chronic stable angina). Another possible mechanism is that amlodipine inhibits vascular smooth muscle carbonic anhydrase I activity causing cellular pH increases which may be involved in regulating intracelluar calcium influx through calcium channels.

EXPERIMENTAL

Olmesartan Medoxomil and Amlodipine Besylate (purity >99.5%) were produced from the SuraLaboratories Ltd., Hyderabad, India. Methanol (Merck Ltd, Mumbai, India) was of

HPLC grade. Analytical grade di-potassium hydrogen phosphate, tri ethylamine, phosphoric acid and sodium hydroxide were produced from S.D. Fine chemicals Ltd, Mumbai, India. The water for HPLC was purchased from Qualigen fine chemicals, Mumbai, India. Injectable dosage form was purchased from local medical store. All the other chemicals used were of analytical grade.

HPLC Instrumentation and analytical conditions

HPLC system (Schimadzu LC-20AD System)equipped with a pump and a PDA detector was used in this study. For data acquisition and processing EMPOWER software was employed. The chromatographic analysis was performed on ahypersil C18 (250mm X 4.6mm X 5µ). The column temperature was maintained at 30^0C. Isocratic elution was performed using a mobile phase of methanol: OPA pH3.0 (40:60, v/v) at a flow rate of 1mL/min. The injection volume was 10µL and the UV detection wavelength was 254nm.

PREPARATION OF BUFFER SOLUTION

Accurately weigh 0.1 ml of ortho phosphoric acid and transfer into a 100ml beaker then add 100 ml HPLC grade water to make up the volume. Mix it properly to get a homogeneous solution.

PREPARATION OF MOBILE PHASE

Ortho phosphoric acid: Methanol (60:40).

Prepared ortho phosphoric acid solution is taken to the required quantity which is called buffer solution and the required amount of the methanol is taken which is called solvent in a beaker both together comprises the mobile phase.

PREPARATION OF THE OLMESARTAN MEDOXOMIL AND AMLODIPINE BESILATE STANDARD AND SAMPLE SOLUTION

Preparation of Olmesartan Medoxomil Standard Solution

Accurately weigh and transfer 50mg of olmesartan medoxomil into 100 ml of volumetric flask and add 20ml of water and sonicate to dissolve. Dilute to volume with water and mix.

Transfer each 2.0 ml of olmesartan medoxomil standard solution into a 100ml volumetric flask, dilute to volume with water and mix.

Preparation of Amlodipine Besilate Standard Solution

Accurately weigh and transfer 50mg of amlodpine besilate into 100 ml of volumetric flask and add 20ml of water and sonicate to dissolve. Dilute to volume with water and mix.

Transfer each 2.0 ml of amlodpine besilatestandard solution into a 100ml volumetric flask, dilute to volume with water and mix.

PREPARATION OF SAMPLE SOLUTION

Commercially available 20 tablets are weighed and powdered equivalent to the 50mg of amlodpine besilate and 50mg of olmesartan medoxomil into a 100ml volumetric flask, add about 20ml of water and sonicate for 30min with intermediate shaking (maintain the sonicator bath temperature between 20-25◦C). Make up to the volume with water and mix. Filter a portion of the solution through 0.45µm membrane filter and discard first few ml of the filtrate.

Transfer each 2.0 ml of above sample solution into a 100ml volumetric flask, dilute to volume with water and mix.

RESULTS AND DISCUSSION

Method development: Premilinary studies were carried out inorder to optimize a suitable method for simultaneous determination of Olmesartan Medoxomil and amlodipine besylate in pharmaceutical dosage form. Trail runs were performed by using C8 and C18 reversed-phase columns, several mobile phase compositions and different flowrates for separation of both drugs with good chromatographic parameters (resolution, symmetry, tailing factor etc,). A hypersil C18 (150mm X 4.6mm X 5µ) using a mobile phase consisting of 0.1% Orthophosphoric acid: Methanol (60:40) at a flowrate of 1mL/min and a detection wavelength of 254nm afforded with the best separation with well-resolved and sharp peaks of both tge drugs. The separation was carried out on an isocratic mode and the injection volume was 10µL.

Method Validation: After method development, validation of the test method was performed in terms of following parameters: linearity and range, accuracy and percentage recovery, precision, limit of detection (LOD), limit of quantification (LOQ) and robustness.

Linearity and range: The linearityof the method was evaluated by analyzing working solutions (calibration standards) of containing 50,75,100, 125 and 150µg/ml for both the

drugs i.e Olmesartan Medoxomil and Amlodipine besylate. The plots of peak areas versus concentrations were linear in the range from 50 to 150µg/ml for both Olmesartan Medoxomil and Amlodipine besylate. The results implied that the method developed was linear over the specified range.

Accuracy and percentage recovery: Recovery studies were carried out at three different concentration levels (50,100 and 150%) in order to check the accuracy of the assay method. The study was performed three times (n=3). The average recovery of Olmesartan Medoxomil was found 101% and of Amlodipine Besylate was found 98%, which indicates the accuracy of the method for determination.

Precision: The relative standard deviation for precision was observed that not more than 2%. The %RSD was observed was 0.66 for Olmesartan Medoxomil and 0.91 for Amlodipine Besylate.

SYSTEM SUITABILITY

Solution of standard sample and placebo were prepared as per test procedure. Equilibrate the column with mobile phase for not less than 30minutes at a flow rate of 1ml/min.

Separately inject 10µL of blank, standard solution (5 times) and sample solution into the chromatographic system.

Tailing factor for the peaks due to olmesartan medoxomiland Amlodipine besylatein standard solution should not be more than 2.0.Theoretical plates for the olmesartan medoxomiland Amlodipine besylatepeaks in standard solution should not be less than 2500.

CONCLUSION

The developed method was successfully applied for simultaneous estimation of Olmesartan medoxomil and Amlodipine besylate in pharmaceutical dosageform. The proposed method was found to be simple, accurate and precise. The method was free from interferences due to excipients present in the formulatiom. Therefore, this method may be useful for routine analysis of Olmesartan medoxomil and Amlodipine besylatein pharmaceutical dosageform.

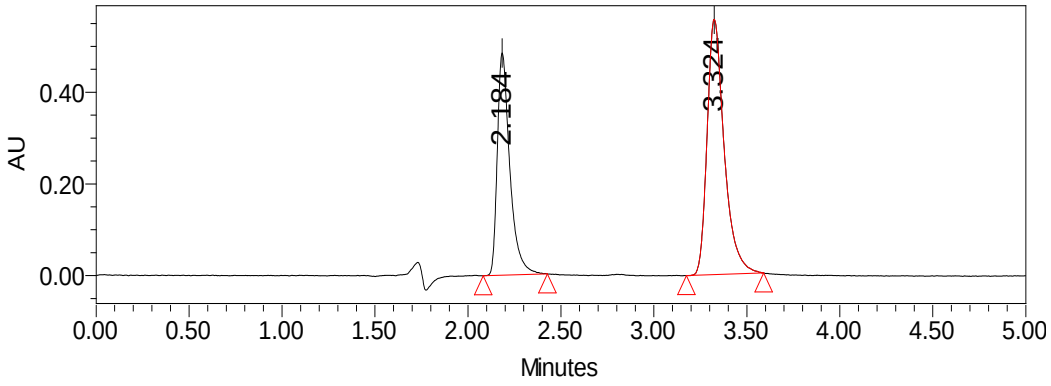

Fig 1: Chromatogram for optimized method.

Table 1: Summary of validation data for Olmesartan medoxomil.

S.NO	PARAMETER	RESULT	ACCEPTENCE CRITERIA
1	System suitability Theoretical plates Asymmetry Retention time %RSD	 13972 1.23 3.3 min 0.6	 Not less than 2000 Not more than2
2	Specificity a) Blank interference b) Placebo interference	Specific	Specific
3	Method precision(%RSD)	0.66	Not more than 2.0%
4	Linearity parameter Intercept Correlation coefficient(r^2)	50-150 mcg/ml 10802 0.999	Not less than 0.999
5	Accuracy Mean % recovery	101	97 - 103%
6	Robustness a) Flow rate variation b) Temperature variation	All the system suitability parameters are within the limits.	

Table 2: Summary of Validation data for Amlodipine Besylate.

S.NO	PARAMETER	RESULT	ACCEPTENCE CRITERIA
1	System suitability Theoretical plates Asymmetry Retention time %RSD	 8760 1.26 2.1 min 1.0	 Not less than 2500 Not more than 2
2	Specificity c) Blank interference d) Placebo interference	Specific	Specific
3	Method precision(%RSD)	0.91	Not more than 2.0%
4	Linearity parameter	50-150 mcg/ml	Not less than 0.999

	Intercept Correlation coefficient(r^2)	43363 0.999	
5	Accuracy Mean % recovery	98	97 - 103%
6	Robustness c) Flow rate variation d) Temperature variation	All the system suitability parameters are within the limits.	

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