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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF AMLODIPINE BESYLATE AND LOSARTAN POTASSIUM IN MULTIDRUG MARKETED FORMULATION

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Abstract

The present work describes a validated RP-HPLC method for simultaneous estimation of Amlodipine Besylate (AB) and Losartan Potassium (LP) in Multidrug Marketed Formulation. The method was performed using Triethylamine 0.02% pH adjusted to 2.1 using O-phosphoric acid: acetonitrile: methanol [50:30:20v/v] as a mobile phase. The chromatographic separation was performed on PRONTOSIL C18 column (250 mm × 4.6 mm I.D. 5µm partical size). Areas were recorded at 238 nm for both the drugs and retention time was found at 4.72 min and 9.19 min for Amlodipine and losartan respectively at 1.2 ml/min flow rate. The selected chromatographic conditions were found to quantitatively determine amlodipine and losartan in a combined dosage form without any interference from matrix. Linearity was found over the range of 25-75 µg/ml for amlodipine and 250-750 µg/ml for losartan. The proposed method was found to be fast, accurate, precise, specific, rugged and robust and can be used for simultaneous analysis of these drugs in combined formulations.

Keyword: Amlodipine, Losartan, Simultaneous estimation, RP-HPLC.

Introduction

Amlodipin Besylate (AB) is a 3-ethyl 5-methyl 2- [(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4 - dihydropyridine-3,5-dicarboxylate (Fig.1), represent the class of long - acting blocker, employed in the management of anti-hypertension, angina [1-6]. The individual determination of AB has been carried out in formulations by UV-Spectrophotometric, HPLC, and Liquid Chromatographic - tandem Mass Spectrometry [21-24]. Second drug,

Losartan Potassium (LP) is chemically [2 – butyl -4–chloro –1- ({ 4 -[2 - (2 H - 1, 2, 3, 4 - tetrazol - 5 - yl) phenyl] phenyl } methyl) - 1 H - imidazol - 5 - yl] methanol (Fig.2). It represents the class of angiotensin II receptor antagonist which is commonly employed for the treatment of high blood pressure (hypertension) [1-6]. The individual determination of LP has been carried out in formulation by UV - Spectrophotometric, Derivative Spectrophotometric, HPLC, Liquid Chromatography, Electro spray ionization tandem Mass Spectrometry, HPTLC [17-20].

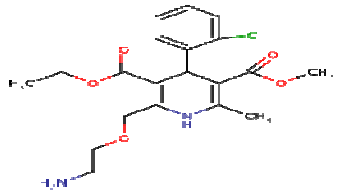

Fig.1: Structure of Amlodipine Besylate

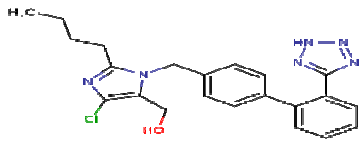

Fig.2: Structure of Losartan Potassium

Literature review did not reveal any method for simultaneous determination of AB and LP in combined pharmaceutical dosage form. So, we decided to work towards development and validation of simple, sensitive, accurate, precise, rugged and economic method for simultaneous determination of these drugs in combined dosage forms. The present work describes a validated reverse phase HPLC method for simultaneous determination of these drugs in multidrug marketed formulation.

Materials and Method

Instrumentation

A Water's HPLC (600), PDA Detector, Auto sampler & 600, Column - PRONTOSIL C18 column (250 mm × 4.6 mm I.D. 5µm partical size). Software- Empower Pro, pH meter (Hanna), Borosilicate volumetric flasks (10ml, 25ml, 100ml), CY 104 micro Analytical balance (Citizen) and Ultrasonic bath.

CHEMICALS AND MATERIALS:

Authentic samples of Amlodipine Besylate (AB) and Losartan Potassium (LP) were supplied by Lupin Research Park Ltd., Pune. Water (HPLC grade), Triethylamine, Acetonitrile (HPLC grade), Methanol (HPLC grade), Orthophosphoric acid.

CHROMATOGRAPHIC CONDITIONS: PRONTOSIL C18 column (250mm ×4.6mm i.d., 5µm particle size). Mobile phase-Water (0.02%TEA) (pH2.1) O-phosphoric acid: Acetonitrile: Methanol [50:30:20v/v]. Flow rate: 1.2 ml/min. Mobile Phase was degassed before use. Detection wavelength: 238 nm, The injection volume: 20 µl.

PREPARATION OF THE MOBILE PHASE:

The mobile phase was water (0.02%TEA) (pH2.1) O-phosphoric acid: Acetonitrile: Methanol [50:30:20v/v]. The mobile phase was filtered through 0.45 µm membrane filter and was degassed before use.

PREPARATION OF SOLUTIONS:

STANDARD STOCK SOLUTION:

SOLUTION A (50 µG/ML):

Weighed accurately 5mg of Amlodipine besylate working reference standard and transferred carefully into a 100ml volumetric flask. Added 75ml of Acetonitrile and sonicated for 15min, cooled to room temperature and diluted upto 100ml with Acetonitrile and Mixed well.

SOLUTION B (500µG/ML):

Weighed accurately 50mg of Losartan Potassium working reference standard and transferred carefully in to a 100ml volumetric flask. Added 75ml of Acetonitrile and sonicated for 15min, cooled to room temperature and diluted upto 100ml with Acetonitrile and Mixed well.

MIXTURE STANDARD SOLUTION:

Diluted 5ml of Solution (A) and 5ml Solution (B) upto 100ml with Acetonitrile.

DETERMINATION OF WAVELENGTH OF MAXIMUM ABSORBANCE:

The standard solution of AB and LP were scanned in the range of 200 - 400 nm against mobile phase as a blank. From the overlain spectrum the wavelength selected for the estimation of drugs were 238nm as an isoabsorptive point. The wavelength selected was 238 nm as both the drugs showed significant absorbance at this wavelength.

CHROMATOGRAPHIC METHOD:

PRE-TREATMENT OF COLUMN:

PRONTOSIL C18 column was properly washed with acetonitrile (HPLC grade previously filtered with membrane filter and degassed properly) for 30 min at 1.2 ml/min of flow rate.

CHROMATOGRAPHIC SEPARATION:

As the system is auto samplier, 20 µl of each working standard solutions or sample solution was injected into the column at 1.2 ml/min flow rate. The Peaks of AB and LP were detected at 238nm and retention times were found to be 4.72 and 9.19 minutes respectively.

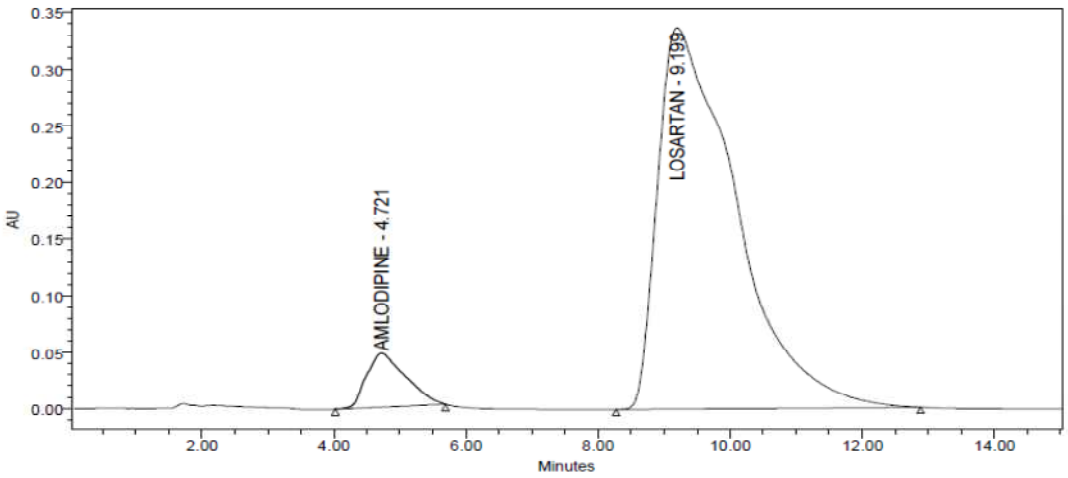

Fig.3: Typical Chromatogram of Amlodipine Besylate&Losartan Potassium.

CALIBRATION CURVE OF STANDERED AB AND LP:

A calibration curves were plotted over a concentration range of 25-75µg/ml for AB and 250-750 µg/ml for LP. Accurately measured standard stock solutions of AB (0.1, 0.2, 0.3, 0.4 and 0.5 ml) and LP (0.5, 1.0, 1.5, 2.0 and 2.5 ml) were transferred to a series of 10 ml borosilicate volumetric flasks and the volume in each flask was adjusted to 10ml with mobile phase. The resulting solution was injected into the column and the peak area obtained at retention time 4.72 and 9.19minutes and flow rate 1.2 ml/min were measured at 238nm for AB and LP respectively. Calibration curves were constructed for AB and LP by plotting peak area versus concentration at 238nm. Each reading was average of three determinations.

QUANTITATION OF AB AND LP IN FORMULATION

20 tablets were weighed accurately and transferred and the average fill weight was determined. Weighed content equivalent to 10 tablets were transferred into 100ml volumetric flask and 50ml of diluents was added,

mixed well and sonicated for 15 minutes with intermittent shaking. The solution was allowed to cool at room temperature. The solutions were diluted upto 100ml with Acetonitrile. Equal volumes of standard and sample solution (20 µL) were injected into the column and chromatographed using optimized chromatographic conditions. The corresponding chromatograms were recorded and area of each peak for AMLO and LOSAR was measured at 238 nm. Amount of AMLO and LOSAR in sample (mg) was calculated by comparing the mean peak area of sample with that of standard.

Method Validation:

System Suitability

System suitability parameter is establishes to ensure that the validity of the analytical method is maintained whenever used. Typical variations are the stability of analytical solution, different equipment, and different analyst. In case of liquid chromatography typical variations are the pH of the mobile phase, the mobile phase composition, different lots or supplier of columns, the temperature (20 ± 30 C) and flow rate.

Precision

The precision is measure of either the degree of reproducibility or repeatability of analytical method. It provides an indication of random error. The precision of an analytical method is usually expressed as the standard deviation, Relative standard deviation or coefficient of variance of a series of measurements.

Ruggedness

Ruggedness is degree of reproducibility of result under a variety of condition.

Linearity and Range

The linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation proportional to the concentration of analyte in samples within a given range. The range of analytical method is the interval between upper and lower level of analyte including levels that have been demonstrated to be determining with precision and accuracy using the method. The linear response of AB and LP were determined by analyzing five independent levels of the calibration curve in the range of 25- 75µg/ml for AB and 250-750µg/ml for LP. Result should be expressed in terms of Correlation co-efficient.

Specificity: The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. In the case of assay, demonstration of specificity requires that it can be shown that the procedure is unaffected by the presence of impurities or excipients.

Accuracy

It is defined as closeness of agreement between the actual (true) value and analytical value and obtained by applying test method for a number of times. Accuracy may often be expressed as % Recovery by the assay of known, added amount of analyte. It is measure of the exactness of the analytical method. The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the capsules (OME 2 µg/ml and DICLO 5 µg/ml) with three different concentrations of standards (OME 1, 2, 3 µg/ml and DICLO 5, 10, 15 µg/ml).

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Results and Discussion

The proposed method can determine AB and LP in multidrug dosage form and the validity of this method was confirmed in accordance with the ICH guidelines. In proposed method retention times were recorded at 4.72min and 9.19min at 1.2 ml/min. flow rate for AB and LP respectively. The calibration graphs for AB and LP were constructed by plotting the area versus their corresponding concentrations, good linearity was found over the range 25-75µg/ml for AB and 250-750µg/ml for LP. The proposed Result of System Suitability Parameter of AB and LP are shown in Table 1. The proposed method gives good recovery data shown in Table 2. Method has been applied to the assay of AB and LP in pharmaceutical dosage form showing Results in Table 3. The validity of the method was further assessed by applying the standard addition technique. The results obtained indicate that the additives present do not interfere with analysis of the studied mixtures (Table 2). The validation parameters are reported in Table 4.

Table No-1: System Suitability Studies.

PARAMETERS	AMLODIPINE BISYLATE	LOSARTAN POTASSIUM
Theoretical plates	2182.34	3306.79
Resolution Factor	4.478	
Tailing factor	1.46	1.9

Table No-2: Recovery data for Amlodipine Besylate and Losartan Potassium.

Recovery level		Amlodipine Besylate				
	Area	Amount added	Amount recovered	% Recovery	Average recovery	% RSD
50%	1291102	25	2.5659	102.4	101.88	0.178897
	1282517	25	2.5488	101.95		
	1274243	25	2.5323	101.29		
100%	2495883	50	4.960	99.20	101.15	1.56025
	2565285	50	5.098	101.96		
	2498621	50	4.965	99.31		
150%	3745598	75	7.443	99.25	99.073	0.30016
	3726059	75	7.405	98.73		
	3745321	75	7.443	99.24		
	Overall				100.70	0.6797

Recovery level		Losartan Potassium				
	Area	Amount added	Amount recovered	% Recovery	Average recovery	% RSD
50%	17727250	25	25.48	101.85	101.94	0.0766
	17751122	25	25.52	101.99		
	17749324	25	25.51	101.98		
100%	34778739	50	49.42	98.81	99.093	0.2693
	34492312	50	49.58	99.13		
	34569514	50	49.69	99.34		
150%	51953202	75	74.69	99.57	99.53	0.3034
	51559088	75	74.12	99.81		
	51786304	75	74.45	99.21		
	Overall				100.187	0.2164

Table No-3: Assay of Amlodipine Besylate and Losartan Potassium.

Sr. No.	AMLODIPINE BISYLATE		LOSARTAN POTASSIUM	
	Assay (mg)	Assay % of LC	Assay (mg)	Assay % of LC
1	4.965	99.30	49.99	99.99
2	4.952	99.52	49.83	99.83
3	4.963	99.47	49.69	100.02
Average	4.96	99.43	49.83	99.94
SD	0.007	0.115	0.152	0.102
% RSD	0.1402	0.115	0.301	0.102

Table No-4: Validation Parameters of Amlodipine Besylate and Losartan Potassium.

Parameters	**Amlodipine Besylate**	**Losartan Potassium**
System suitability		
Tailing Factor	1.46	1.9
No. Of theoretical plate	2182.34	3306.79
Retention Time	4.7	9.1
Precision*(%RSD)	0.31%	0.25%
Ruggedness*		
Day to Day (% RSD)	1.07%	0.40%
Accuracy* (%Recovery)		
50%	101.95%	101.99%
100%	99.20%	99.13%
150%	99.25%	99.57%
Robustness*		
Change in flow rate(%RSD)	1.53%, 1.01%	0.08%, 0.33%
Change in Organic phase(%RSD)	1.66%, 0.84%	1.4%, 0.08%
Changein pH(%RSD)	0.43%, 1.73%	0.97%, 0.19%
Linearity*		
Range (µg/ml)	25µg/ml-75ug/ml	25µg/ml-750µg/ml
Correlation co-efficient (R^2) Slope	0.992 58558	0.997 157149

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