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RP- HPLC Method for Simultaneous Estimation of Losartan potassium and Amlodipine besylate in Tablet Formulation

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ABSTRACT: This work is concerned with application of simple, precise, accurate and reproducible reverse phase high performance liquid chromatographic (RP-HPLC) method for simultaneous estimation of Losartan potassium (LP) and Amlodipine besylate (AB) on RP C-18 Column (Microsorb-MV 100-5, 250 x 4.6 mm) using 0.02% triethylamine in water: acetonitrile (60:40), pH adjusted to 2.5 with O- phosphoric acid as mobile phase at a flow rate of 1.0 ml/min and the detection wavelength was 226 nm. The retention time for LP and AB was found to be 2.32 and 10.10 min, respectively. Proposed method was validated for precision, accuracy, linearity range, robustness and ruggedness.
KEY WORDS: Losartan potassium, Amlodipine besylate, Reverse Phase High Performance Liquid Chromatography.

INTRODUCTION:

Losartan Potassium (LP), 2-n-butyl-4-chloro-1-[p-(o-1H-tetrazol- 5-ylphenyl) benzyl]-imidazole-5 methanol monopotassium salt is a highly selective, orally active, non-peptide angiotensin II receptor antagonist indicated for the treatment of hypertension. It has a more potent active metabolite EXP3174 (II, 2-n-butyl-4-chloro-1-[2-(1H-tetrazol-5 yl) biphenyl- 4-yl) methyl] imidazole-5-carboxyl acid)[1]. The determination of Losartan has been carried out in tablets by HPLC, capillary electrophoresis and super-critical fluid chromatography[2,3], in urine by gas chromatography- mass spectrometry[4] and, simultaneously with its active metabolite in biological fluids, by HPLC[5-10].

Amlodipine Besylate (AB), chemically, 2-[(2-aminoethoxy) methyl]- 4- (2-chlorophenyl) -1, 4-dihydro- 6-methyl-3, 5-pyridinedicarboxylic acid 3-ethyl, 5-methyl ester, is an anti-hypertensive and an antianginal agent in the form of the besylate salt, Amlodipine besylate. It is not official in any Pharmacopoeia. Various analytical methods have been reported for the assay of Amlodipine besylate[11] in pure form as well as in pharmaceutical formulations. They include high performance liquid chromatography,[12-17] reversed phase high performance liquid chromatography,[18-21] high performance thin layer chromatography,[22-25] gas chromatography,[26] gas chromatography–mass spectrometry,[27] liquid chromatography with tandem mass spectrometry[28] and fluorimetry,[29] derivative spectroscopy,[30,31] simultaneous multicomponent mode of analysis and difference spectrophotometry[32-34].

By using this method, no RP-HPLC study on simultaneous estimation of Losartan and Amlodipine in tablet dosage form in pharmaceutical preparations has been found in literature survey. There was very few HPLC methods have been reported[35] for simultaneous estimation of Losartan and Amlodipine in pharmaceutical dosage form, which prompted to pursue the present work. The objective of the present work is to develop and validate new analytical methods for simultaneous determination of Losartan Potassium and Amlodipine Besylate in tablet dosage form. This communication forms the first report of simple, sensitive and reproducible method for the simultaneous estimation of Losartan Potassium and Amlodipine Besylate from combined dosage form.

MATERIAL AND METHOD:

Chemicals and Reagents

LP and AB were obtained as gift samples from Lupin Research Park Ltd., Pune and Shreya Life Sciences Pvt. Ltd. Aurangabad (M. S.) respectively. Water (HPLC grade), acetonitrile (HPLC grade), triethylamine and ortho-phosphoric acid were of reagent grade. The pharmaceutical preparations of combination of Losartan and Amlodipine that is Alsartan AM tablet (ARISTO, Mumbai). The commercial formulation of LP and AB is

available in ratio of 10:1 {Alsartan AM (5/0.5 mg)} as tablet.

Instrumentation

A Gradient HPLC PU 2080 Plus (JASCO) with UV-2075 Plus detector and RP-C18 column was used. A Rheodyne injector with a 20 µl loop was used for the injection of sample. The HPLC system was equipped with Borwin software for data processing.

Chromatographic Condition

The mobile phase containing triethylamine in water: acetonitrile (60:40), pH adjusted to 2.5 with O-phosphoric acid was found to resolve LP and AB. Ortho-phosphoric acid was used for pH adjustment of buffer. The mobile phase was filtered on a 0.45 micron membrane filter and then ultrasonicated for 30 min. The flow rate was set to 1.0 ml/min. Both drugs showed good absorbance at 226 nm, which was selected as wavelength for further analysis. All determinations were performed at constant column temperature (28 ± 2^0 C).

Preparation of Stock Solutions

Standard stock solutions containing Losartan Potassium (LP) and Amlodipine besylate (AB) were prepared individually by dissolving 50 mg of LP and quantity of AB equivalent to Amlodipine base 5 mg separately in 80 ml of methanol. It was then sonicated for 10 minutes and the final volume of both the solutions were made up to 100 ml with methanol to get stock solutions containing 500 µg/ mL of LP and 50 µg/ mL of AB resp.

Calibration curve

Calibration curves were prepared by taking appropriate aliquots of standard LP and AB stock solutions in different 10 ml volumetric flask and diluted up to the mark with mobile phase to obtain final concentrations of 50, 100, 200, 300, 400, 500 µg/ml of LP and 5, 10, 20, 30, 40, 50 µg/ml of AB. Standard solutions (n=6) were injected through 20 µl loop system and chromatograms were obtained using 1.0 ml/min. flow rate. The effluent was monitored at 226 nm. Calibration curve was constructed by plotting average peak area against concentration and regression equation was computed.

Validation of the method

The developed method was validated in terms of linearity, accuracy, specificity, limit of detection, limit of quantification, intra-day and inter-day precision and repeatability of measurement.

Sample Preparation

A total of 20 tablets were accurately weighted and triturated with glass mortar and pestle. An amount equivalent to one tablet (containing 5 mg of LP and 0.5 mg of AB) was transferred to a 100ml volumetric flask; 50 ml of mobile phase was added and the flask was kept in an ultrasonic bath for 10 min. The volume was made up to mark and the solution was filtered through 0.2 micron nylon membrane filter. The final volume of both the solutions were made up to 100 ml with mobile phase to get stock solutions containing 500 µg/ mL of LP and 50 µg/ mL of AB resp. The diluted solution was analyzed under optimized chromatographic conditions and chromatogram is depicted in fig. No.1 and 2.

RESULT AND DISCUSSION:

To develop a precise, accurate and suitable RP- HPLC method for the simultaneous estimation of LA and AB, different mobile phases were tried and the proposed chromatographic conditions were found to be appropriate for the quantitative determination. The results obtained by the assay of marketed formulation are summarized in Table.1. System suitability tests were carried out as per USP XXIV and parameters are summarized in Table.2**.**

Method Validation[36]

The proposed HPLC method was validated as per ICH guidelines.

Specificity

The peak purity of LP and AB were assessed by comparing the retention time (TR) of standard LP and AB. Good correlation was obtained between the retention time of standard and sample of LP and AB.

Linearity

Linearity was studied by preparing standard solutions at different concentration levels. The linearity range for LP and AB were found to be 50- 500 µg/ml and 5-50 µg/ml, respectively. The regression equation for LP and AB were found to be $y = 73190x - 2053395$ and $y = 5177x - 13541$ with coefficient of correlation, (r) 0.9999 and 0.9999, respectively.

Precision

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in sample preparation. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise. Results are shown in Table 3.

Accuracy (Recovery studies)

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 50%, 100% and 150%. Known amounts of standard LP and AB were added to pre-analyzed samples and were subjected to the proposed HPLC method. Results of recovery studies are shown in Table 2.

Robustness of method

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate, pH and mobile phase ratio on the retention time and tailing factor were studied. The method was found to be unaffected by small changes like ± 0.1 change in pH, ± 0.1 change in flow rate and ± 1 change in mobile phase.

CONCLUSION:

The proposed method is simple, sensitive and reproducible and hence can be used in routine for simultaneous determination of LP and AB in bulk as well as in pharmaceutical preparations. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The RSD for all parameters was found to be less than one, which indicates the validity of method and assay results obtained by this method are in fair agreement. The developed method can be used for

routine quantitative simultaneous estimation of LP and AB in multicomponent pharmaceutical preparation.

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Table no. 1. Result of LP and AB in marketed formulation (n=6).

Marketed Formulation	**Drug**	**% Amount found ± SD**	**% RSD**
Alsartan AM tablet (ARISTO, Mumbai).	LP	100.35 ± 0.75	0.74
	AB	100.46 ± 0.67	0.67

S.D: Standard deviation, RSD: Relative standard deviation

Table no. 2. System Suitability Parameters

Parameter		**LP**	**AB**
Linearity range (µg/ml)		50- 500	5- 50
Correlation coefficient		0.9999	0.9999
Slope*		73190.74	5177.58
Retention time* (min.)		2.32	10.10
Resolution factor		1.14	1.24
Tailing factor*		1.45	1.50
Accuracy* (Recovery studies)	50%	99.94 ± 0. 45	98.23 ± 0.37
	100%	99.12 ± 0.68	99.21 ± 0.75
	150%	99.35 ± 0.27	99.38 ± 0.41

*Average of six readings ± Standard deviation.

Table No.3: Statistical Evaluation of Precision of developed method.

Drug	**Intraday**	**Interday**
LP	% Mean ± SD	
	99.96 ± 0.14	100.04 ± 0.22
AB	100.09 ± 0.53	100.00 ± 0.19

S.D: Standard deviation.

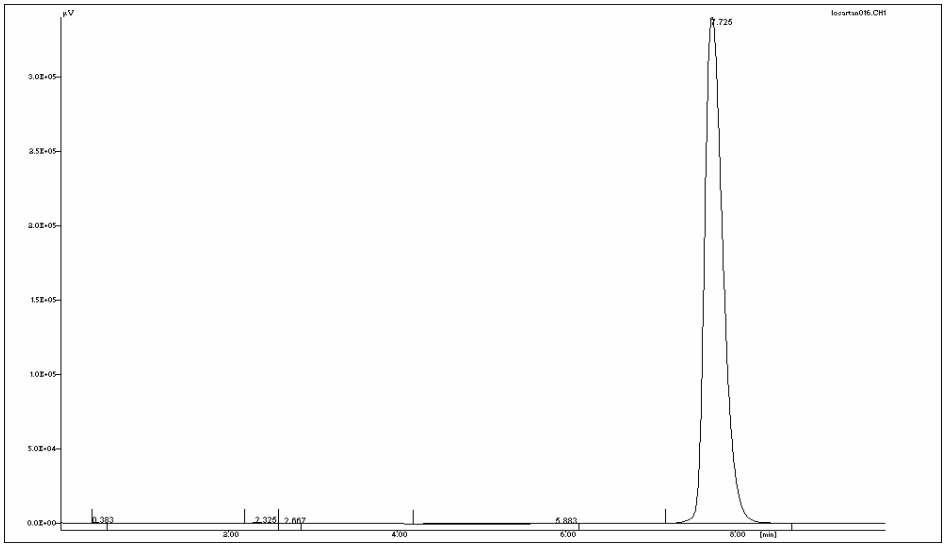

FIG.-1 Typical chromatogram of LP (RT=10.40 min).

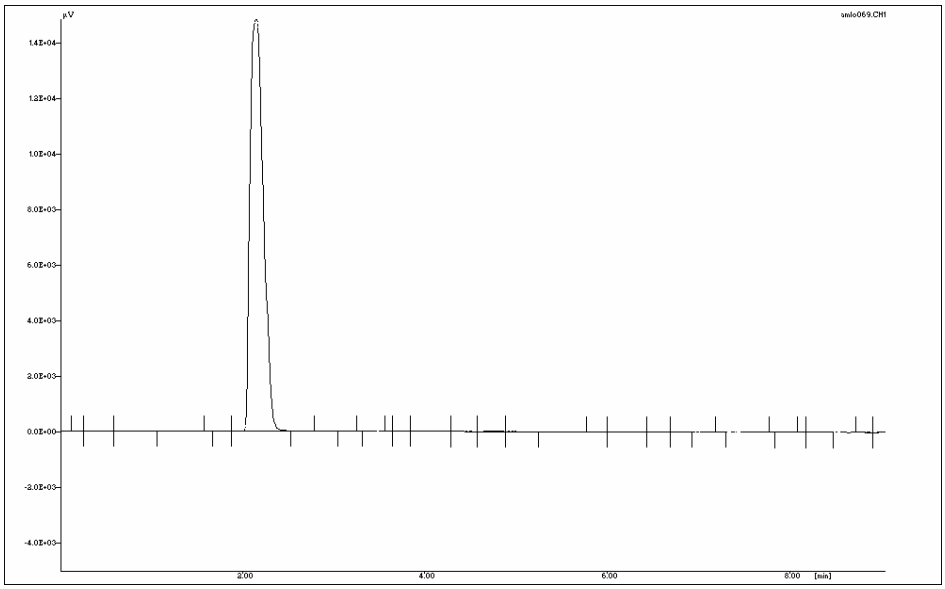

FIG.-2 Typical chromatogram of AB (RT= 2.32 min).

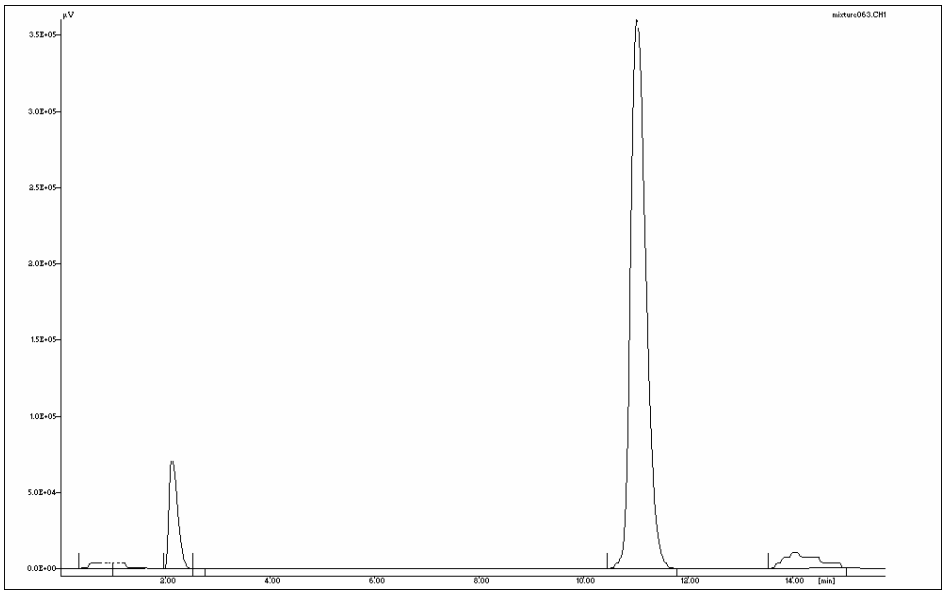

FIG.-3 Typical chromatogram of LP (RT=2.32 min) and AB (RT= 10.10 min).

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