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A Validated RP-HPLC Method For Simultaneous Estimation Of Losartan Potassium And Amlodipine In Pharmaceutical Formulation

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ABSTRACT

A simple, selective, rapid, precise and economical reverse phase high pressure liquid chromatographic method has been developed for the simultaneous estimation of losartan potassium and amlodipine besylate from pharmaceutical formulation. The method was carried out on a Princeton SPHER C_{18} (15 cm x 4.6 mm i.d., 5 μ) column with a mobile phase consisting of acetonitrile: dipotassiumorthophosphate (adjusted to pH 7.5 using orthophosphoric acid) (50:50 v/v) at a flow rate of 0.7 ml/min. Detection was carried out at 239 nm. Diazepam was used as an internal standard. The retention time of losartan, amlodipine and diazepam was 5.36, 3.76 and 15.47 min, respectively. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation and solution stability. The proposed method can be used for the estimation of these drugs in combined dosage forms.

KEYWORDS

RP-HPLC;
Losartan and amlodipine.

INTRODUCTION

Losartan is chemically designated as (1-((2'-(2H-tetrazol-5-yl)biphenyl-4-yl)methyl)-2-butyl-4-chloro-1H-imidazol-5-yl)methanol. Losartan is an angiotensin II receptor antagonist drug used mainly to treat high blood pressure (hypertension). Losartan was the first angiotensin II receptor antagonist to be marketed. Amlodipine is a 2-[(2-amino ethoxy) methyl]-4-(2 chloro phenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylicacid-3-ethyl 5-methyl ester. And it's a calcium channel blocker which is used as antihypertensive and anti-anginal agent. It has long elimination half life and large volume of distribution.

EXPERIMENTAL

Reagents and chemicals

Acetonitrile HPLC grade was procured from Qualigens (India) Ltd, Mumbai. Dipotassiu morthophosphate and orthophosphoric acid AR grade were procured from Qualigens fine chemicals, Mumbai. Water HPLC grade was obtained from a Milli-QRO water purification system. Reference standards of losartan and amlodipine are procured from Medrich steri lab, Bangalore and diazepam was procured from Til India Pharmaceuticals Ltd, Banglore.

Apparatus and chromatographic conditions

Chromatographic separation was performed on a Shimadzu® liquid chromatographic system equipped with a LC-2010A-vp solvent delivery system (pump), ultra violet detector, class-VP 6.01 data station was applied for data collecting and processing (Shimadzu, Japan). PrincetonSPHER C_{18} (15 cm x 4.6 mm i.d., 5μ) was used for the separation, mobile phase of a mixture of acetonitrile and Dipotassium orthophosphate (adjusted to pH 7.5 using orthophosphoric acid) (50:50 v/v) was delivered at a flow rate of 0.7 ml/min with detection at 239 nm. The mobile phase was filtered through a 0.2 μ membrane filter and degassed. The injection volume was 50 μl; Analysis was performed at ambient temperature.

Preparation of standard solutions

Standard stock solutions of 1.0 mg/ml losartan, amlodipine and diazepam were prepared separately using a mixture of water and acetonitrile (1:1 v/v). From the standard stock solution, mixed standard solution was prepared to contain 25 μg/ml of losartan, 5.0 μg/ml of amlodipine and 100.0 μg/ml of diazepam as internal standard.

Preparation of sample solutions

Ten Tablets, each containing 25 mg of losartan and 5.0 mg amlodipine of were weighed and finely powdered; a quantity of powder equivalent to 0.2 mg of losartan and 2.0 mg of amlodipine was weighed and transferred to a sintered glass crucible. To this 5.0 ml of 1.0 mg/ml solution of diazepam was added and the drugs were extracted with three quantities, each of 20 ml of mixture of acetonitrile and water (1:1 v/v). The combined extracts were made up to 100 ml with mobile phase and further dilutions were made to get a concentration of 25 μg/ml of losartan, 5.0 μg/ml of amlodipine (theoretical value) and 100.0 μg/ml of diazepam as internal standard and this solution was used for the estimation.

Assay method

With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solution was injected and the chromatogram was recorded. The retention time of losartan, amlodipine and diazepam was found to be 5.36, 3.76 and 15.47 min, respectively. This procedure was repeated for the sample solution obtained from the formulation. The response factor (peak area ratio of standard peak area and internal standard peak area) of the standard solution and sample solution were calculated. The concentration of the drugs were calculated (TABLE 1) using following formula

$$\text{Concentration of drugs} = \frac{\text{Response factor of the sample}}{\text{Response factor of the standard}} \times \text{Concentration of standard}$$

TABLE 1: Results of analysis of formulation and recovery studies

Drug	Amount mg/ tab		% Label claim*	% Recovery*
	Labelled	Found*		
Losartan	25 mg	24.58	98.32	99.56
Amlodipine	5 mg	4.97	99.40	98.12

*** Average of six determinations, mean ± standard deviation**
REPACE A (Sun pharmaceuticals) each tablet containing 25 mg of Losartan and 5 mg of Amlodipine

RESULTS AND DISCUSSION

Estimation of losartan and amlodipine in dosage forms

Estimation of losartan and amlodipine in dosage forms by RP-HPLC method was carried out using optimized chromatographic conditions. The standard and sample solutions were prepared. The chromatograms were recorded. The typical chromatogram of sample solution is shown in figure 1. Detection

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was done at 239 nm. The overlaid UV spectrum of losartan and amlodipine is shown in figure 2. The peak area ratio of standard and sample solutions was calculated. The assay procedures were repeated for six times and mean peak area and mean weight of standard drugs was calculated. The percentage of individual drugs found in formulations, mean, standard deviation in formulations were calculated and presented in TABLE 1. The results of analysis shows that the amounts of drugs were in good agreement with the label claim of the formulations.

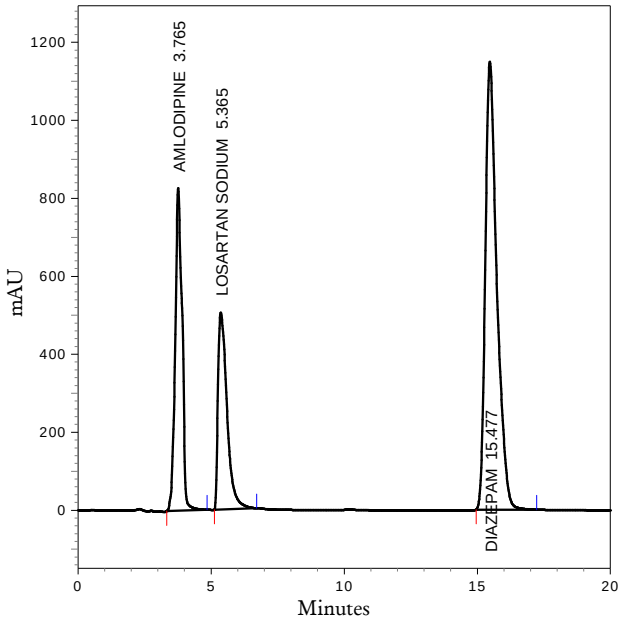

Figure 1: Typical chromatogram of sample solution

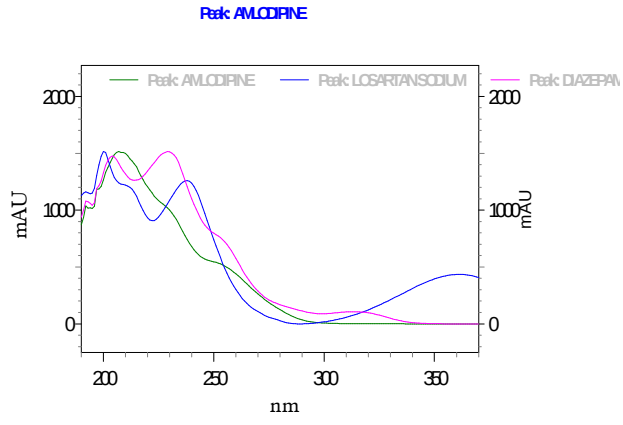

Figure 2: Overlaid UV spectrum of losartan and amlodipine

Method validation

1. Accuracy and precision

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in TABLE 1. From the data obtained, added recoveries of standard drugs were found to be accurate.

The precision of the method was demonstrated by inter day and intra day variation studies. In the intra day studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated and presented in TABLE 2. In the inter day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drugs peaks and percentage RSD were calculated and presented in TABLE 2. From the data obtained, the developed RP-HPLC method was found to be precise.

2. Linearity and range

The linearity of the method was determined at five concentration levels ranging from 5 to 30 µg/ml for losartan and 1.0 to 12.0µg/ml for amlodipine (TABLE 3). The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was y = 0.0041 for Losartan and y = 0.0032 for amlodipine. The results shows that an excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above. The calibration curves are shown in figure 3 and 4.

3. Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for losartan and amlodipine was found to be 10 ng/ml and 5.0 ng/ml, respectively. The LOQ is the small-

TABLE 2: Intraday and interday precision studies

Intraday studies				Interday studies				
RF* of amlodipine	Mean (% RSD*)	Rf of losartan	Mean (% RSD)	Day	Rf of amlodipine	Mean (% RSD)	Rf of losartan	Mean (% RSD)
				Day 1	0.0063	0.0599	0.0568	0.0265
					0.0535		0.0157	
					0.0534		0.0198	
					0.0655	0.0059	0.0236	0.0171
					0.0638		0.0168	
0.0621		0.0168		Day 2	0.0639	0.0554	0.0588	0.0387
0.0535		0.0268			0.0435		0.0167	
0.0691		0.0156			0.0534	0.0081	0.0164	0.0273
0.0589	0.0607	0.0157	0.01834		0.0623		0.0253	
0.0612		0.0166			0.0538		0.0253	
	0.0069		0.00477	Day 3	0.0539	0.5583	0.0664	0.0322
					0.0635		0.1588	
					0.0638		0.0468	0.0232
					0.0563	0.0050	0.0157	
					0.0538		0.0165	

*Rf-Response factor, % R.S.D - Relative standard deviation

TABLE 3: Linearity and range

Losartan		Amlodipine	
Concentration	Response factor	Concentration	Response factor
5	0.0211	1	0.0033
10	0.0423	3	0.0101
15	0.0635	5	0.0168
20	0.0847	7	0.0235
25	0.1059	10	0.0336
30	0.1271	12	0.0403

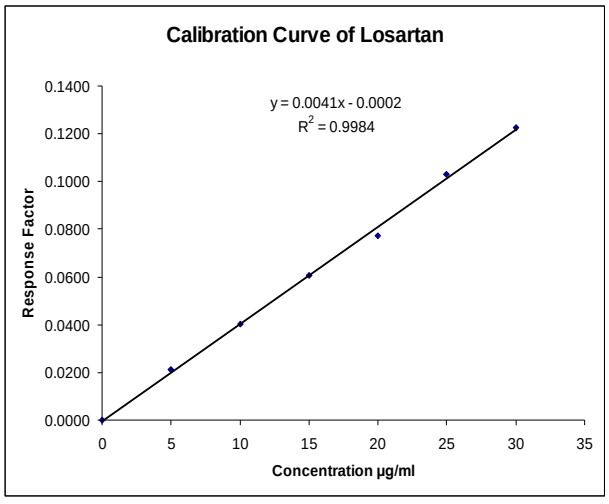

Figure 3: Calibration curve of losartan

est concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ was 30 ng/ml and 15 ng/ml for losartan and amlodipine, respectively (TABLE 4).

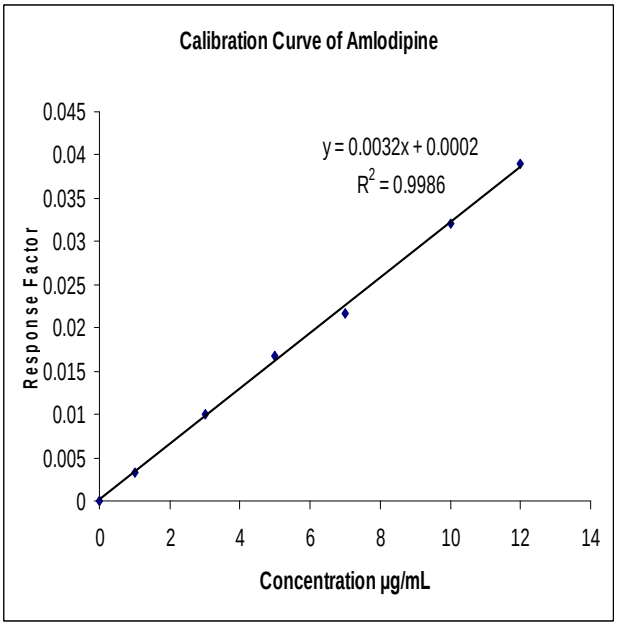

Figure 4: Calibration curve of amlodipine

TABLE 4: System suitability studies

S.No.	Parameters	Losartan	Amlodipine
1	Theoretical plate/meter	6217	4072
2	Resolution factor	3.49	
3	Asymmetric factor	1.54	0.95
4	LOD (ng/ml)	10 ng/ml	5 ng/ml
5	LOQ (ng/ml)	30 ng/ml	15 ng/ml

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4. Ruggedness and robustness

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC ($LC2010A_{HT}$), Agilent HPLC and Water's Breeze HPLC by different operators using different columns of similar type like Hypersil C_{18}, Phenomenex Gemini C_{18} and Hichrom C_{18}. Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed are rugged and robusted.

6. Solution stability

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 5h at room temperature. The results show that for both solutions, the retention time and peak area of losartan and amlodipine remained almost unchanged (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for atleast 5h, which was sufficient to complete the whole analytical process.

5. System suitability studies

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions. (TABLE 4). The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 % standard deviation range during routine performance of the method.

CONCLUSION

The proposed RP-HPLC method for the simultaneous estimation of losartan and amlodipine in combined dosage forms are accurate, precise, linear, rugged, robusted, simple and rapid. Hence the present RP-HPLC method is suitable for the quality control of the raw materials, formulations and dissolution studies.

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