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A LIQUID CHROMATOGRAPHIC MEHTOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF AMLODIPINE BESYLATE AND LOSARTAN IN PURE AND MARKETED FORMULATIONS

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ABSTRACT

A novel, simple, accurate, stability indicating reverse phase liquid chromatographic method was established and validated for the simultaneous quantification of amlodipine besylate and losartan in pure and marketed formulations. Quantification of the selected drugs was done with a C18 column [Agilent column. 250mm × 4.6 mm]using mobile phase of composition Acetonitrile and phosphate buffer (60:40 v/v, pH 3). The flow rate was 0.8 ml/min and the effluents were monitored at 226 nm. The retention time of Amlodipine besylate and Losartan were 2.9 min and 4.2 min respectively. The method was found to be linear over a range of 10-50 µg/ml for Amlodipine besylateand 2-10 µg/ml for Losartan. The established method proved as reproducible one with a %RSD value of less than 2 and having the robustness and accuracy within the specified limits. Assay of marketed formulation was determined and find with 99.08% and 99.87% for Amlodipine besylate and Losartan respectively. The stressed samples were analyzed and this proposed method was found to be specific and stability indicating as no interfering peaks of degradation compounds and excipients were noticed. The method was validated according to the guidelines of International Conference on Harmonization (ICH) and was successfully employed in the estimation of commercial formulations. This liquid chromatographic method can be applied for the qualitative and quantitative determination of selected drugs by the modern chemist.

KEYWORDS: Amlodipine, Losartan, RP-HPLC, Stability and Method validation.

INTRODUCTION

Amlodipine besylate (AMB) is chemically 3-Ethyl 5-methyl 2-(2-aminoethoxymethyl)4-(2-chlorophenyl)-1,4-dihydro-6-methylpyridine-3,5dicarboxylate mono benzene sulphonate used in the treatment of hypertension and congestive heart failure. Losartan potassium(LOS) is chemically described as 2-butyl-4-chloro-1{[2'-(1H-tetrazol-5-yl)][1,1'-biphenyl]-4-yl]methyl}-1H-imidazol (Fig.1.a) and is mainly used to treat high blood pressure(hypertension) as it is a competitive antagonist and inverse agonist of Angiotensin-II receptor.

Extensive literature survey proved that very few methods were reported for the determination of Amlodipine besylate and Losartan by RP-HPLC.[2,12] So we attempted to develop an accurate, rapid, precise, stable, sensitive and economically viable liquid chromatographic method for the simultaneous determination of selected drugs in the present research.

MATERIALS AND METHODS

Equipment used

The chromatographic separation was performed on Agilent 1120 compact liquid chromatographic system integrated with a variable wavelength programmable UV detector and a Rheodyne injector equipped with 20µl fixed loop. A reverse phase C18[Agilent ODS UG 5 column, 250mm × 4.5 mm]was used. Lab India 3000^{+} double beam UV visible spectrophotometer and Axis AGN204-PO electronic balance were used for spectrophotometric determinations and weighing purposes respectively.

Reagents and chemicals

Pharmaceutical grade pure Amlodipine besylate and Losartan gift samples were procured from Mylan Laboratories, Hyderabad. Marketed formulation Tablets with dose of 5mg of Amlodipine besylate and 50mg of Losartan were procured from local market. (Mfd. by Intas). HPLC grade Acetonitrile and Water were procured from Merck specialties private limited, Mumbai.

Chromatographic conditions

Kromasil 100-5C_{18} column [250mm x 4.6mm] was used for the chromatographic separation at a detection wave length of 226 nm. Mobile phase of composition Acetonitrile and Phosphate buffer pH 3 in a ratio of

60:40 v/v was selected for elution and same mixture was used in the preparation of standard and sample solutions. Flow rate was adjusted to 0.8 ml/min and the injection volume was 20µl.

Preparation of Mobile phase

Phosphate buffer pH 3 was prepared by dissolve 0.136 gm of Potassium dihydrogen phosphate and 2 ml of Triethyl amine in 80ml of HPLC grade water and adjusts the pH to 3.0 with orthophosphoric acid and sufficient water was added to produce100 ml filtered through 0.45µ membrane filter and sonicated for 20 minutes.

Preparation of Standard solutions

25mg each of Amlodipine besylate and Losartan were accurately weighed and transferred into two 25ml volumetric flasks, dissolved using mobile phase and the volume was made up with the same solvent to obtain primary stock solutions A (Nebivolol) and B (Losartan) of concentration 1000µg/ml of each drug. From the primary stock solutions, 5ml and 1ml were pipette out from A and B respectively, transferred to a 10ml volumetric flask and the volume was made up with the mobile phase to obtain final concentrations of 50 µg/ml and 10µg/ml of Amlodipine besylate and Losartan respectively and this solution is (working stock solution A).

Preparation of Sample Solution

Twenty tablets of Amlodipine besylate and Losartan were weighed and crushed. Tablet powder equivalent to 5mg of Amlodipine besylate and 0.5mg of Losartan was weighed accurately and transferred to a 25ml volumetric flask. The content was dissolved with 10ml of mobile phase and then sonicated for 15min. The volume was made up with the mobile phase and filtered with 0.45µ membrane filter and sonicated for 20min. 0.5ml of this solution was pipette out and transferred to a 10ml volumetric flask and the volume was made up with the mobile phase to obtain a concentration of 60µg/ml of Amlodipine besylate and 22.5 µg/ml of Losartan (working stock solution B).

Optimization of RP-HPLC method

The HPLC method was optimized with an aim to develop a simultaneous estimation procedure for the assay of Amlodipine besylate and Losartan. For the method optimization, different mobile phases were tried, but acceptable retention times, theoretical plates and good resolution were observed with Acetonitrile, Phosphate buffer pH 3 (60:40 v/v) using Kromasil 100-5C_{18} column [250mm x 4.6mm].

Validation of the RP-HPLC method

Validation of the optimized method was performed according to the ICH Q2 (B) guidelines.

System suitability

System suitability was carried out with five injections of solution of 100% concentration having 50 µg/ml of Amlodipine besylate and 10 µg/ml of Losartan in to the chromatographic system. Number of theoretical plates (N) obtained and calculated tailing factors (T) were reported in table 1.

Linearity

For the determination of linearity, appropriate aliquots were pipette out from working stock solution A to a series of 10ml volumetric flasks and volume was made up with the solvent to obtain concentration ranging from 10-50µg/ml of Amlodipine besylate and 2-10µg/ml of Losartan. Each solution was injected in triplicate. Calibration curves were plotted with observed peak areas against concentration followed by the determination of regression equations and calculation of the correlation coefficients. The calibration curves for Amlodipine besylate and Losartan were shown in figure 3 and figure 4 their corresponding linearity parameters were given in table 2.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were calculated from the slope(s) of the calibration plot and the standard deviation (SD) of the peak areas using the formulae LOD = 3.3 σ/s and LOQ = 10 σ/s. The results were given in table 2.

Precision

The repeatability of the method was verified by calculating the %RSD of six replicate injections of 100% concentration (50 µg/ml of Amlodipine besylate and 10µg/ml of Losartan) on the same day and for intermediate precision % RSD was calculated from repeated studies on different days. The results were given in table 3.

Accuracy

To ensure the reliability and accuracy of the method recovery studies were carried out by standard addition method. A known quantity of pure drug was added to pre-analyzed sample and contents were reanalyzed by the proposed method and the percent recovery was reported. The results were given in table 4.

Specificity

Specificity of a method was determined by testing standard substances against potential interferences. The method was found to be specific when the test solution was injected and no interferences were found because of the presence of excipients. The optimized chromatogram of Amlodipine besylate and Losartan without any interference was shown in figure 2.

Robustness

Robustness of the method was verified by altering the chromatographic conditions like mobile phase composition, wave length detection, flow rate, etc. and the % RSD should be reported. Small changes in the operational conditions were allowed and the extent to which the method was robust was determined. A

deviation of ±2nm in the detection wave length and ±0.2ml/min in the flow rate, were tried individually. A solution of 100% test concentration with the specified changes in the operational conditions was injected to the instrument in triplicate. %RSD was reported in the table 5.

Assay of Marketed Formulations

20µl of sample solution of concentration 50 µg/ml of Amlodipine besylate and 10 µg/ml of Losartan was injected into chromatographic system and the peak responses were measured. The solution was injected three times in to the column. The amount of drug present and percentage purity was calculated by comparing the peak areas of the standards with that of test samples. A typical chromatogram for assay of marketed formulation was shown in figure 5 and the obtained values were reported in the table 6.

Stability Studies

Acid degradation studies

Prepared each 1mg/ml stock solution of Amlodipine besylate and Losartan by using mobile phase as solvent, and then filtered through 0.45µm membrane filter paper. Stock solutions of 5 ml and 1ml of Amlodipine besylate and Losartan stock solution was transferred into 10ml volumetric flask and added 1 ml of 0.1N HCL and diluted to volume with mobile phase. The resultant solution was injected into the system; there was no acid degradation products were found the obtained chromatogram was shown in figure 6.

Alkaline degradation studies

Prepared each 1mg/ml of stock solution with Amlodipine besylate and Losartan then filtered through 0.45µm membrane filter paper. Stock solutions of 5 ml and 1ml of Amlodipine besylate and Losartan stock solution was transferred into 10ml volumetric flask and added 1 ml of 0.1N NaOH and diluted to volume with mobile phase. The obtained non interfered chromatogram was represented in figure 7.

Oxide degradation studies

Prepared each 1mg/ml of stock solution of Amlodipine besylate and Losartan then filtered through 0.45µm membrane filter paper. Stock solutions of 5 ml and 1ml of Amlodipine besylate and Losartan stock solution was transferred into 10ml volumetric flask and added 1 ml of H_2O_2 and diluted to volume with mobile phase. In this investigation no identifiable oxidative degradants were found and the chromatogram was shown in figure 8.

Thermal degradation studies

Prepared each 1mg/ml of stock solution with Amlodipine besylate and Losartan and then filtered through 0.45µm membrane filter paper. Stock solutions of 5 ml and 1ml of Amlodipine besylate and Losartan10ml volumetric flask and diluted to volume with mobile phase and kept for 60min at 60^0c in hot air oven. From the obtained chromatogram it was proved that the selected samples were stable against thermal conditions. The chromatogram was shown in figure 8.

RESULTS AND DISCUSSION

After a number of trials with mobile phases of different composition, Acetonitrile, Phosphate buffer pH 3.0 in the ratio 60:40 v/v was selected as mobile phase because of better resolution and symmetric peaks. Amlodipine besylate and Losartan were found to show appreciable absorbance at 226nm when determined spectrophotometrically and hence it was selected as the detection wavelength. An optimized chromatogram showing the separation of Amlodipine besylate and Losartan at different R_Ts was shown in figure 2.

System suitability was carried out by injecting 5 replicate injections of 100% test concentration, number of theoretical plates, HETP and resolution were satisfactory. The chromatograms confirm the presence of Amlodipine besylate and Losartan at 2.9min and 4.2min respectively without any interference. The parameters were given in table 1.

Concentration range of 10-50µg/ml for Amlodipine besylate and 2-10µg/ml of Losartan were found to be linear with correlation coefficients 0.999 and 0.999 for Amlodipine besylate and Losartan respectively. The results were given in table 2.

The limits of detection for Amlodipine besylate and Losartan were found to be 0.16µg/ml and 10.33µg/ml respectively and the limit of Quantitation were 0.49µg/ml and 1.01µg/ml respectively. Values were represented in table 2.

The proposed method was found to be precise and reproducible with %RSD of 0.87 and 1.14 for Amlodipine besylate and Losartan respectively. %RSD was reported in table 3.

Accuracy of the method was verified by performing recovery studies by standard addition method. The percent recovery of the standard added to the pre-analysed sample was calculated and it was found to be 98.8% to 99.4% for Amlodipine besylate and 98.6 to 100.8% for Losartan. This indicates that the method was accurate. Values obtained were given in table 4.

The method was found to be robust after changing the conditions like detection wavelength (± 2nm) and flow rate (± 0.2 ml). %RSD was calculated for each variation and reported. Values obtained were given in table 5.

The method was found to be specific for the combination of interest after verifying the chromatograms showing no interference of the excipients present. Hence, the method was well suitable for the estimation of the commercial formulations of the selected combination with a percentage purity of 99.08% for Amlodipine besylate and 99.87% for Losartan. The typical chromatogram for

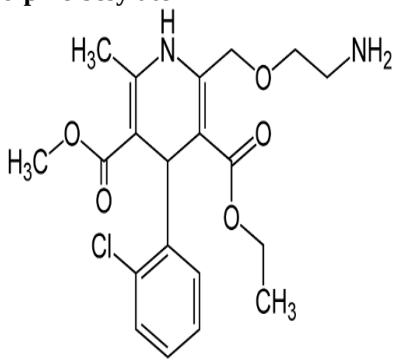
assay of marketed formulations was shown in figure.5 and Values obtained were given in table 6.

Forced Degradation Study

Degradation studies indicated the specificity of developed method in presence of degradation products. Degradation was carried out in combination of two drugs and purity of drug peaks was confirmed by purity angles. Their combination drug products were exposed to acid, base, oxidative and thermal stress conditions. Then found to be no degradable substances presence and proved that the proposed method was stable towards acid, alkali, peroxide and thermal conditions. The obtained values were reported in table 7.

Figures and Tables

a) Amlodipine besylate



b) Losartan

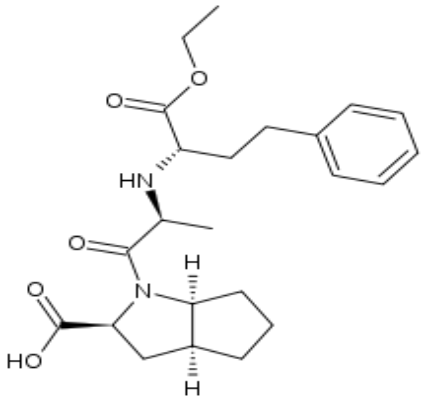


Fig. 1: Chemical Structures of a) Amlodipine besylate and b) Losartan.

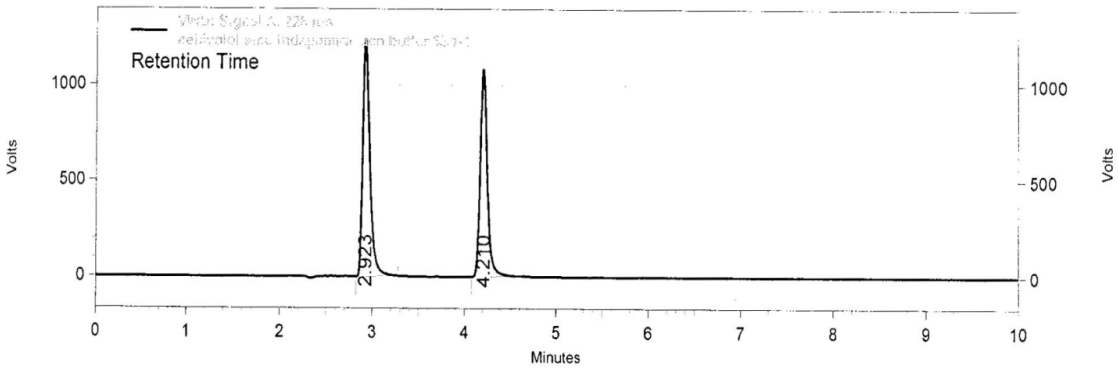


Fig. 2: Optimized chromatogram of Amlodipine besylate and Losartan.

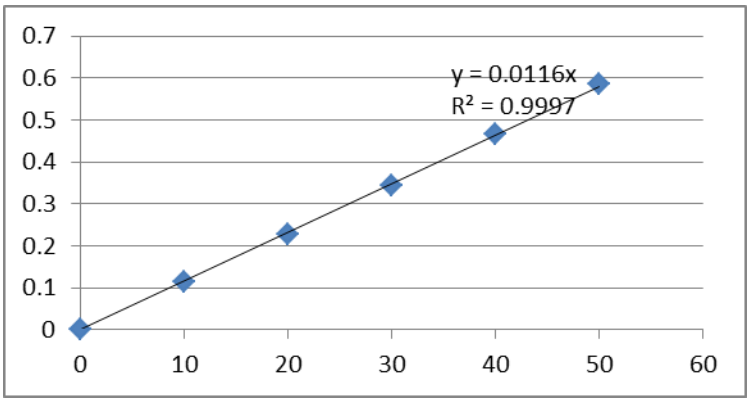


Fig. 3: Calibration plot of Amlodipine besylate.

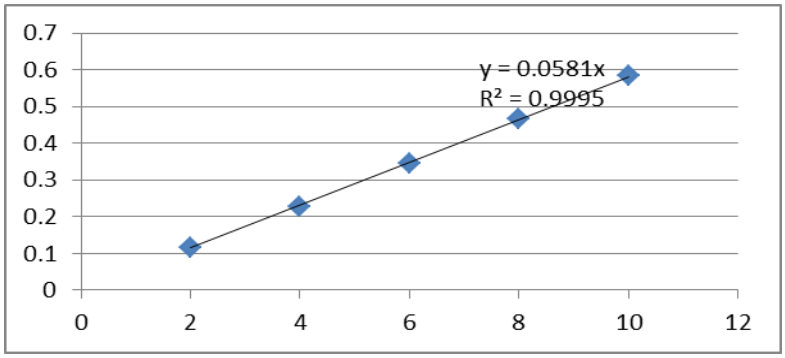


Fig. 4: Calibration plot of Losartan.

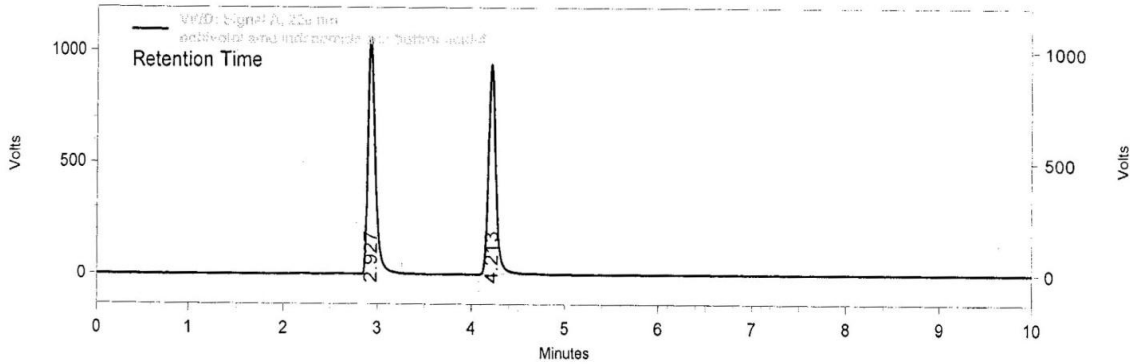


Figure 5: A typical chromatogram for assay of marketed formulation containing 50µg/ml of Amlodipine besylate and 10 µg/ml Losartan.

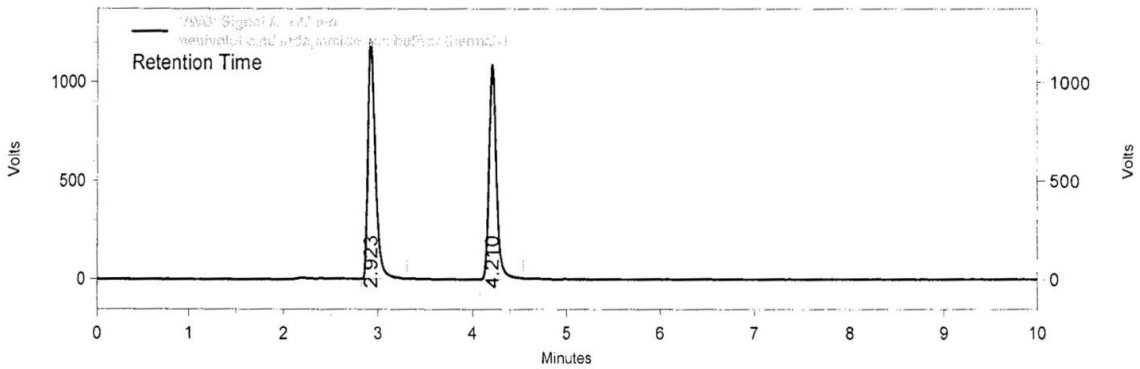


Figure 6: Chromatogram of acid degradation.

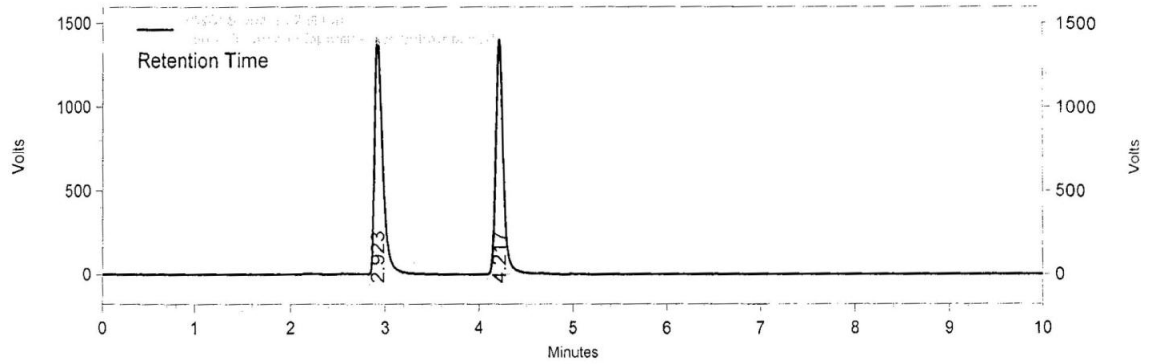


Figure 7: Chromatogram of alkaline degradation.

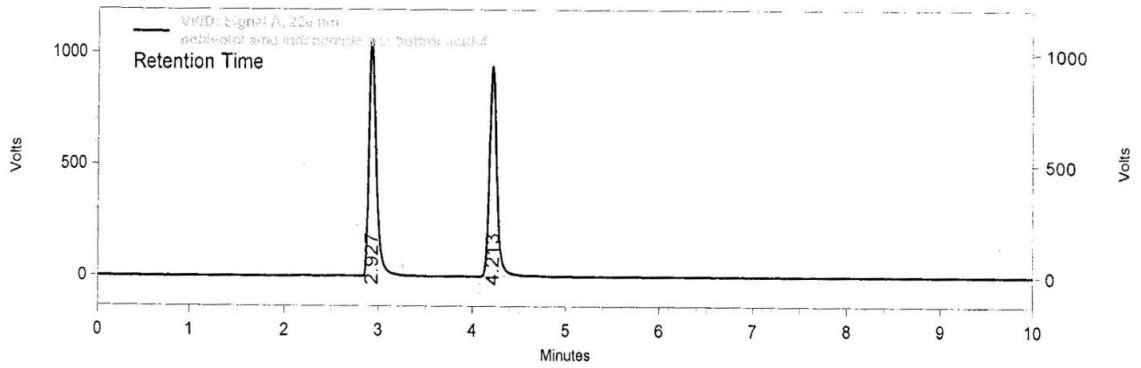


Figure 8: Chromatogram of peroxide degradation.

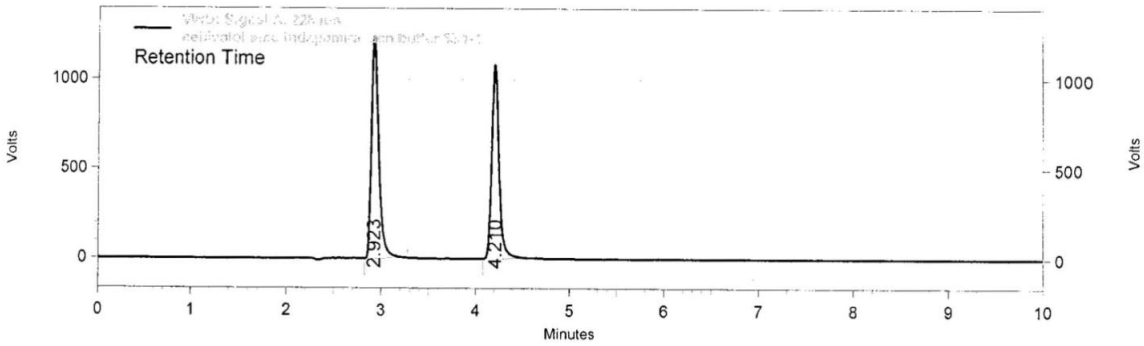


Figure 9: Chromatogram of thermal degradation.

Table 1: System Suitability Parameters.

Parameters	Amlodipine besylate	Losartan
Retention time (min)	2.8	4.2
Theoretical plates (N)	11456	10366
Tailing factor (T)	1.2	1.4
Resolution (R_s)	2.89	

Table 2: Results for Linearity.

Parameters	Amlodipine besylate	Losartan
Slope	0.0122	0.0119
y intercept	0.011+0.0122=0.0232	0.058+0.0119=0.0699
Correlation coefficient r^2	0.999	0.999
Regression Equation	Y=31326351x+0.011	Y=6235614x+0.058
Linearity range	10-50µg/ml	2-10µg/ml
LOD	0.16µg/ml	0.33µg/ml
LOQ	0.49µg/ml	1.01µg/ml

Table 3: Results of Precision.

Drug	Intraday Precision (%RSD)	Interday Precision (%RSD)
Amlodipine besylate	0.69	0.87
Losartan	0.79	1.14

Table 4: Results for Accuracy.

Recovery level	Amlodipine besylate				Losartan			
	Amount Added (µg/ml)		Amount Found (µg/ml)	% Recovery	Amount Added (µg/ml)		Amount Found (µg/ml)	% Recovery
	std	test			std	Test		
80%	20	20	39.6	99.0	2	6	7.93	99.80
100%	30	20	49.7	99.4	4	6	9.86	98.6
120%	40	20	59.3	98.8	6	6	12.1	100.8
Mean recovery	98.8-99.4%				98.6-100.8%			

Table 5: Results for Robustness.

Parameters (n=3)	%RSD	
	Amlodipine besylate	Losartan
Detection wavelength at 228nm	0.93	0.56
Detection wavelength at 224nm	0.72	0.98
Flow rate 0.6ml/min	0.86	0.56
Flow rate 1.0ml/min	0.51	0.48

Table 6: Results for Assay of Marketed formulation.

Drug	Label claim (mg/tab)	Amount recovered	% Amount found in drug
Amlodipine besylate	5	4.67	99.08%
Losartan	1.5	1.42	99.87%

Table 7: Results for Stability studies of Amlodipine besylate and Losartan combined form.

Parameters	Peak area		% of degradation	
	Amlodipine besylate	Losartan	Amlodipine	Losartan
Acid degradation	134401822	93798681	0.125	0.196
Alkali degradation	139591459	926567597	0.112	0.156
Peroxide degradation	135162589	956743021	0.268	0.341
Thermal Degradation	137511860	956556341	0.262	0.192

CONCLUSION

The RP-HPLC method developed and validated allows a simple and fast quantitative determination of Amlodipine besylate and Losartan from their formulations. All the validation parameters were found to be within the limits according to ICH guidelines. The proposed method was found to be specific for the drugs of interest irrespective of the excipients present and the method was found to be simple, accurate, precise, rugged, robust and stable under forced degradation stress conditions. So the established method can be employed in the routine analysis of the marketed formulations.

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