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Development and validation of RP-HPLC method for simultaneous estimation of losartan potassium and amlodipine besylate in synthetic mixture and pharmaceutical dosage form

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ABSTRACT

A precise, simple, accurate, reproducible, rapid, and economic RP-HPLC method has been developed for simultaneous estimation of losartan potassium and amlodipine besylate in synthetic mixture and pharmaceutical dosage form. The components were well separated using Hibar^{R} 250-4.6 Purospher^{R} STAR RP-C18 (5µm) column using 0.1% triethylamine in water: methanol (32:68 v/v) PH of which was adjusted to 3.8 with orthophosphoric acid as mobile phase at a flow rate of 1.0 ml/min. The wavelength was selected at 235 nm using UV detector. The retention time of losartan potassium and amlodipine besylate was found to be 7.650 and 6.417 respectively. The method was validated for system suitability, specificity, linearity, accuracy, precision, ruggedness and robustness as per ICH guidelines and the results were found to be within the limits[16].

KEYWORDS

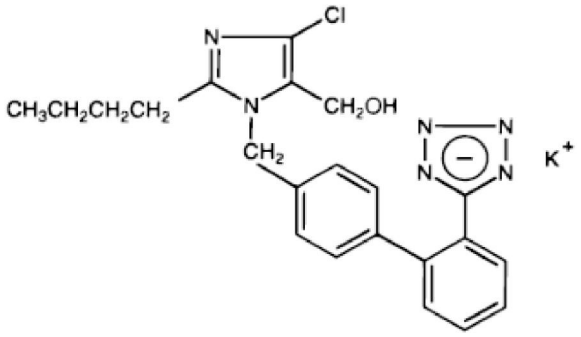
Amlodipine besylate;
Losartan potassium;
Reverse phase high performance liquid chromatography;
Synthetic mixture.

INTRODUCTION

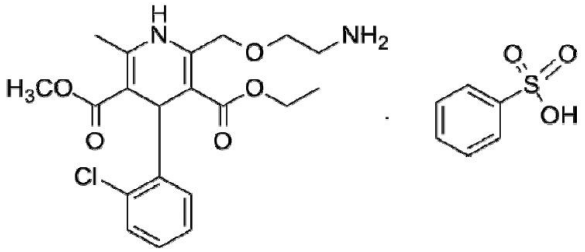
Losartan potassium (LP), or (2-butyl-4-chloro-1-{[2'-(1*H*-tetrazol-5yl)biphenyl]methyl}-1*H*-2imidazol-5-yl) methanol monopotassium salt, is a selective, competitive angiotensin II receptor type 1 (AT1) receptor antagonist, reducing the end organ responses to angiotensin II. Losartan administration results in a decrease in total peripheral resistance (after load) and cardiac venous return (preload)[1-2].

Amlodipine besylate (AB) is chemically -[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid,3-ethyl,5-methyl ester besylate mono benzene sulphonate, is a dihydropyridine calcium antagonist (calcium ion antagonist) that inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. Experimental data suggest that amlodipine binds to both dihydropyridine and nondihydropyridine binding sites. The contractile processes of cardiac muscle and vascular smooth muscle are dependent upon the movement of extracellular calcium ions into these cells through specific ion channels. Amlodipine inhibits calcium ion influx across cell membranes selectively, with a greater effect on vascular smooth muscle cells than on cardiac muscle cells[3-4].

By using this method, no RP-HPLC study with



A) Chemical structure of losartan potassium



B) Chemical structure of amlodipine besylate

Figure 1 : Chemical structures of losartan potassium and amlodipine besylate

the use of 0.1% triethylamine in water: methanol (32:68 v/v) P^H of which was adjusted to 3.8 with ortho-phosphoric acid as mobile phase at a flow rate of 1.0 ml/min has not been reported for simultaneous estimation of losartan potassium and amlodipine besylate in tablet dosage form in literature survey. Reported methods for estimation of losartan potassium and amlodipine besylate are spectrophotometric[5-11], RP-HPLC[12-13] and HPTLC[14-15]. The objective of the present work is to develop and validate new analytical method for simultaneous estimation of losartan potassium and amlodipine besylate in tablet dosage form.

MATERIAL AND METHODS

Chemicals and reagents

Double distilled water, methanol (HPLC grade) was obtained from s d fine-chem. limited, worli Mumbai. Triethylamine (AR grade) was obtained from s d fine-chem. limited, worli Mumbai. Ortho-phosphoric acid (85% pure) was obtained from Merck specialties private limited, worli, Mumbai.

Instrumentation

A Jasco LC-Net-^{22}/ADC with intelligent UV/VIS detector (UV-2075 plus), intelligent HPLC pump (PU-2080 Plus) and RP-C18 column (Purospher^R STAR) was used. A manual syringe injector was used for the injection of sample. The HPLC system was equipped with Borwin software for data processing.

Chromatographic Condition

The mobile phase was prepared using 0.1% triethylamine in water: methanol (32:68), pH of which was adjusted to 3.8 with ortho-phosphoric acid was found to resolve LP and AB. Ortho-phosphoric acid was used for pH adjustment. The degassing of mobile phase was done by sonication for 60 min. The flow rate was set to 1.0 ml/min. Both drugs showed good absorbance at 235 nm, which was selected as wavelength for further analysis. The column temperature was maintained at room temperature.

Preparation of Stock Solutions

Standard stock solutions containing losartan potassium and amlodipine besylate were prepared individually by dissolving 2 mg of drug in 10 ml volumetric flask using mobile phase and volume is make up to the mark. This will give 200 µg/ml of solution. From this 5 ml of solution was pipette out and added in a 10 ml volumetric flask and diluted up to the mark with mobile phase which will give 100 µg/ml

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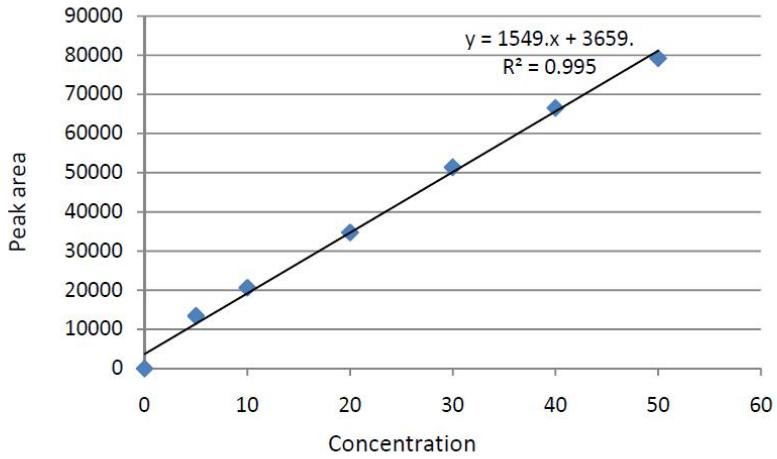
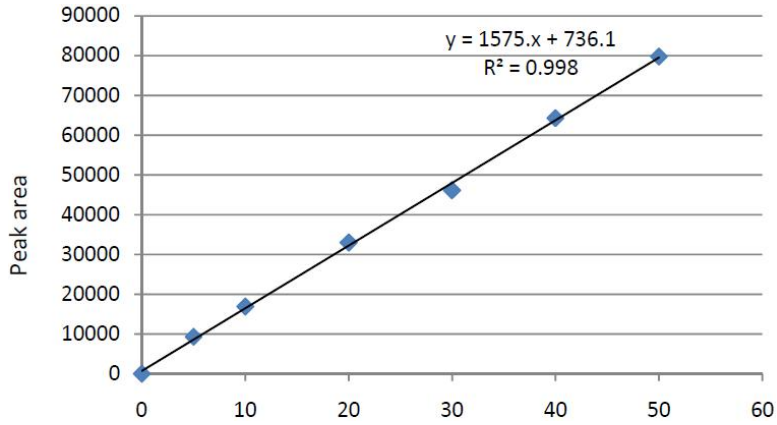


A) Calibration curve and regression equation of losartan potassium



B) Calibration curve and regression equation of amlodipine besylate

Figure 2 : Calibration curve and regression equation of losartan potassium and amlodipine besylate

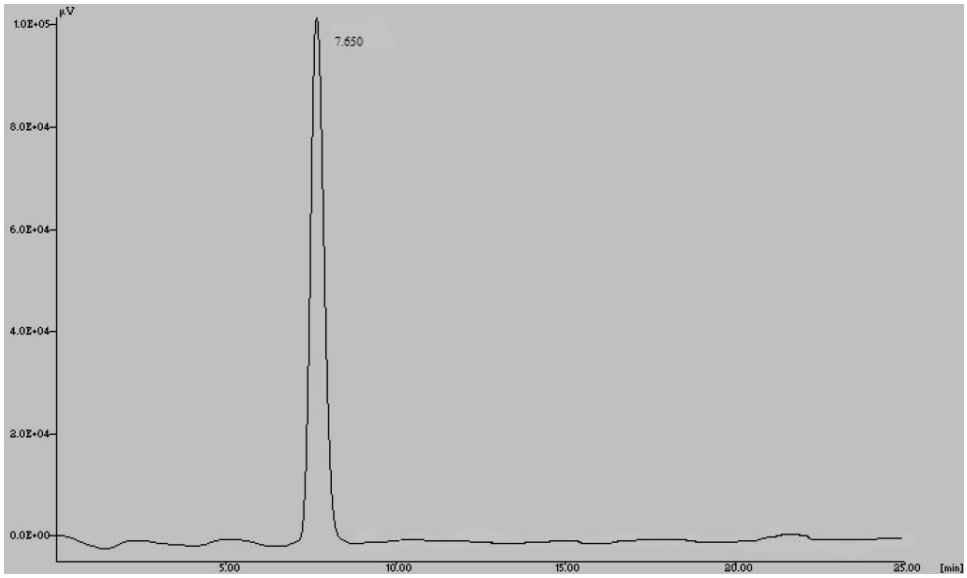


Figure 3 : Chromatogram of losartan potassium sample at λmax (235nm)

solutions respectively for both drugs and used for sample injection.

Sample Preparation

A total of 20 tablets of marketed formulation were accurately weighed and triturated with mortar and pestle (glass). A quantity of powder equivalent

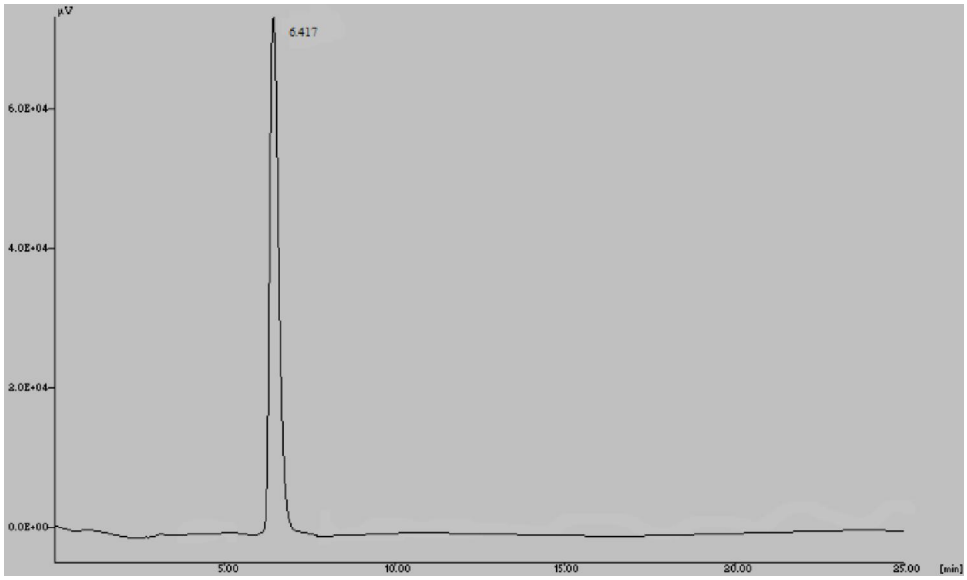


Figure 4 : Chromatogram of amlodipine besylate sample at λmax (235nm)

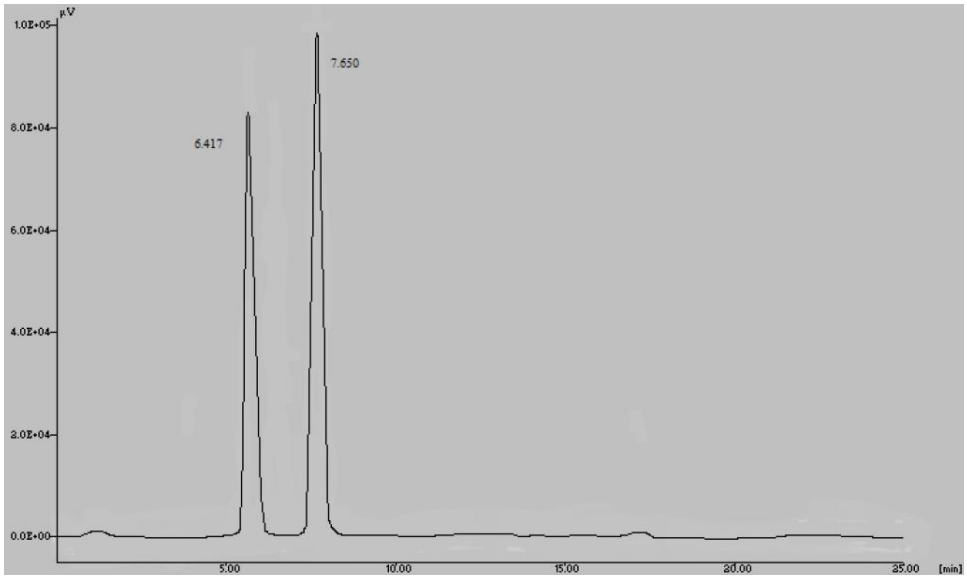


Figure 5 : Chromatogram of synthetic mixture at λmax (235nm)

to 50 mg of losartan potassium and 5mg of amlodipine besylate was accurately weighed and transferred to 100 ml volumetric flask and dissolved in mobile phase and final volume was made up with mobile phase. The sample solution was then filtered through Whatman filter paper no.41. This gives solution of concentration 100 µg/ml. From the above stock solution 5 ml of solution was transferred in 10 ml volumetric flask and was diluted with mobile phase up to 10 ml. This gives solution of 50 µg/ml concentration of losartan potassium and corresponding concentration of amlodipine besylate. This diluted 50 µg/ml solution was analyzed under optimized chromatographic conditions.

Calibration curve

Calibration curves were prepared by taking appropriate volume of standard LP and AB stock solutions in 10 ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 5, 10, 20, 30, 40, 50 µg/ml of LP and AB. Standard solutions were injected through 20 µl loop system and chromatograms were obtained using 1.0 ml/min. flow rate. Calibration curve was made by plotting average peak area against concentration and regression equation was computed.

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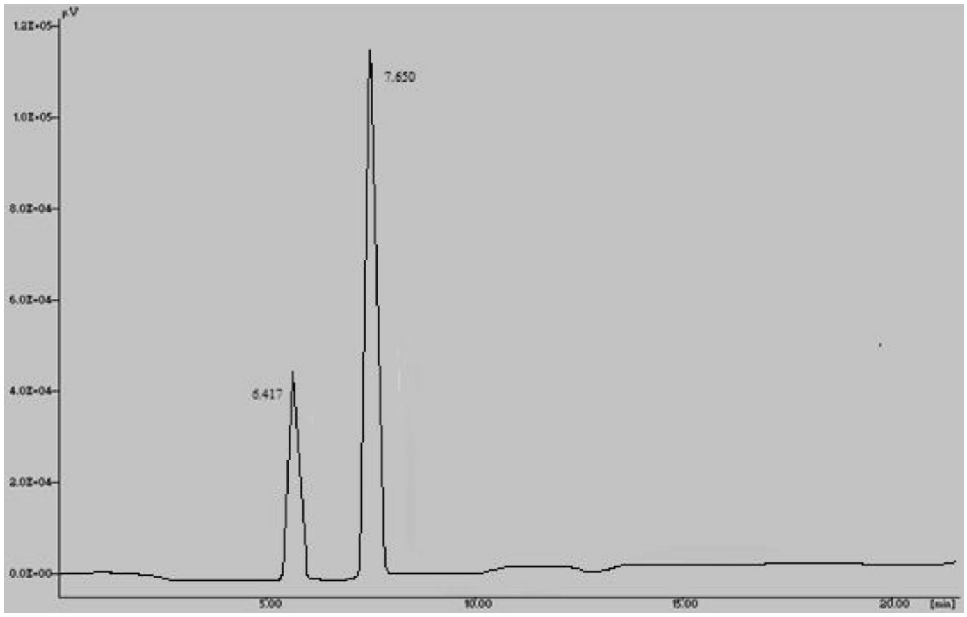

Figure 6 : Chromatogram of tablet sample at λmax (235nm)

TABLE 1 : System suitability data for LP and AB

Parameters	LP	AB
Retention time	7.650	6.417
Theoretical plates	5675.40	5638.44
Capacity	1015.00	1188.00
Resolution	0.00	2.96
Area [µV.Sec]	3897356.975	1845715.929
Asymmetry	1.02	2.13

Method validation[16]

The developed HPLC method was validated as per ICH guidelines.

Specificity

Specificity is the ability of the method to measure the analyte in the presence of other relevant components those are expected to be present in a sample. Specificity of the HPLC method is demonstrated by the separation of the analytes from other potential components such as impurities, excipients or degradants. The purity of LP and AB were analyzed by comparing the retention time of sample with standard LP and AB. Good correlation was obtained between LP and AB.

System suitability

The system suitability parameters with respect to tailing factor, theoretical plates, resolution, capacity, area, and asymmetry are shown in TABLE 1.

Accuracy (Recovery studies)

To check the degree of accuracy of the method, recovery studies were performed in triplicate at 80%, 100% and 120%. 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.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results,

TABLE 2 : Recovery study data for LP and AB

Drugs	Level of recovery (%)	Recovery (%)	± SD	%RSD
LP	80%	100.88	0.638	0.632
	100%	99.09	0.112	0.113
	120%	101.79	0.368	0.361
AB	80%	99.75	0.492	0.493
	100%	100.04	0.605	0.602
	120%	101.56	0.810	0.797

which are directly proportional to the concentration (amount) of analyte in the sample. Linearity was studied by preparing standard solutions at different concentration levels. The linearity range for LP and AB were found to be 5-50 µg/ml for both, respectively. The regression equation for LP and AB were found to be y = 1549x + 3659.0 and y =1575.x + 736.1 with coefficient of correlation, (r^2) 0.995 and 0.998 respectively. Results are shown in TABLE 3

TABLE 3 : Linearity study data for LP and AB

Linearity	LP	AB
Range	5-50 µg/ml	5-50 µg/ml
r^2	0.995	0.998
Slope	1549	1575
Intercept	3659.0	736.1

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample. Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The Interday and intraday precision was determined by assay of the sample solution on the same day and on different days at different time intervals respectively. 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 4.

Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection for an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S respectively. Where S is the slope of the calibration curve and σ is the standard deviation of response. Results are shown in TABLE 5.

TABLE 4 : Intraday and Interday study data for LP and AB

Drug	Intraday (%RSD)	Interday (%RSD)
LP	0.12	0.13
AB	0.45	0.19

TABLE 5 : LOD and LOQ study data for LP and AB

Drug	LOD	LOQ
LP	0.04	0.10
AB	0.0019	0.0027

Robustness of method

The robustness of an analytical procedure 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. 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. Results are shown in TABLE 6.

RESULTS AND DISCUSSION

A RP-HPLC method for the simultaneous estimation of LP and AB was developed. The peaks of losartan potassium and amlodipine besylate are found

TABLE 6 : Robustness study data for LP and AB

Parameters	Changing level	Retention time of LP	Retention time of AB
P^H	3.8	7.650	6.417
	3.5	7.651	6.416
Mobile phase ratio	32:68	7.650	6.417
	30:70	7.650	7.418
Flow rate	1.0 ml/min	7.650	6.417
	1.2 ml/min	7.652	6.417

to be well separated at 7.650 and 6.417 respectively (Figure 7) using 0.1% triethylamine in water: methanol (32:68 v/v) P^H of which was adjusted to 3.8 with ortho-phosphoric acid as mobile phase at a flow rate of 1.0 ml/min with wavelength set to 235 nm. The developed method was validated for various parameters as per ICH guidelines like specificity, precision, accuracy, linearity, specificity, robustness, LOQ and LOD.

The validity and reliability of proposed methods is assessed by recovery studies. Sample recovery for both the methods is in good agreement with their respective label claims, which suggest non interference of formulation additives in estimation. (TABLE 2)

Linearity range of losartan potassium and amlodipine besylate is 5-30 ug/ml. The coefficient of correlation for losartan potassium and amlodipine besylate is 0.995 and 0.998 respectively. (TABLE 3)

The intraday study (% RSD) for LP and AB was found to be 0.12 and 0.45 respectively and interday study (% RSD) for LP and AB found to be 0.13 and 0.19 which are within acceptable limit of d" 2. Hence method is reproducible and the results are shown in (TABLE 4).

The LOD and LOQ values are 0.04, 0.10 ug/ml for LP while LOD and LOQ values are 0.0019, 0.0027 ug/ml for AB. Low values of LOD and LOQ shows good sensitivity of proposed methods. (TABLE 5)

It was observed that on changing different parameters like P^H, mobile phase ratio and flow rate during the robustness study, it does not affect other parameters such as retention time. Hence the method is reliable with variations in the analytical conditions (TABLE 6).

CONCLUSION

The developed rp-hplc method is simple, accurate, sensitive, unique, precise, eco friendly, cost effective, fast and reproducible for simultaneous estimation of losartan potassium and amlodipine besylate in bulk mixture and pharmaceutical dosage form. The method utilizes simple sample preparation, short analysis time and elution is done by isocratic method. The method is validated as per the ICH Guidelines. It is concluded that this method can be adopted by the industries and academic institutions for their combination drug estimation.

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