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Original Article

Method Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Atenolol and Lercanidipine in Bulk and its Pharmaceutical Formulations

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ABSTRACT

A new rapid, precise and sensitive reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed and validated for the estimation of Atenolol and Lercanidipine simultaneously in combined dosage form. The two components Atenolol and Lercanidipine were well resolved on an isocratic method, C18 column, utilizing a mobile phase composition of acetonitrile: methanol: a buffer of 0.02M Potassium dihydrogen phosphate buffer (50:10:40), v/v, pH 5.0) at a flow rate of 1.0 mL/min with UV detection at 226 nm. The retention time of Atenolol and Lercanidipine were 2.0 min and 3.5 min respectively. The developed method was validated for specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and robustness as per ICH guidelines. Linearity for Atenolol and Lercanidipine were found in the range of 208-624 µg/ml and 40-120 µg/ml, respectively. The percentage recoveries for Atenolol and Lercanidipine ranged from 98-101 % and 97-101 %, respectively. The proposed method could be used for routine analysis of Atenolol and Lercanidipine in their combined dosage forms.

Keywords: Liquid chromatography, Atenolol, Lercanidipine, Combined dosage forms, Simultaneous estimation, Validation.

INTRODUCTION

Atenolol (ATL), is chemically (RS)-4-(2-hydroxy-3-isopropylamino propoxy) phenyl acetamide (Figure 1A), is a cardioselective β-blocker. It is reported to lack intrinsic sympathomimetic activity and membrane stabilizing properties. This drug

is used to treat numerous cardiovascular disorders, for example hypertension, angina pectoris, cardiac arrhythmias and myocardial infarction. It is official in USP, IP and BP[1-3].

Lercanidipine is chemically 2-{(3, 3-diphenylpropyl) methylamine}-1, 1-dimethylethyl, methyl-1, 4-dihydro-2, 6-dimethyl-4- (3-nitrophenyl)-3, 5- pyridinedi-carboxylic ester (Figure 1B) with molecular formula $C_{36}H_{41}N_3O_6$. It is a new third generation 1, 4-dihydropyridine calcium channel antagonist used as antihypertensive agent. It is official in Merck Index and Martindale[4-6].

Atenolol alone or in combination with other drugs is reported to be estimated by HPLC in pharmaceutical dosage form[7-17], plasma[18-20], Serum[21], urine[22], UV spectro-photometry[23-31], spectrofluorimetry[32], gas–liquid hromatography[33], Chemometric[34], UPLC[35], HPTLC[36], capillary zone electro-phoresis[37].

Some analytical methods for quantitative determination of Lercanidipine Hydrochloride in pharmaceutical formu-lations are described in literature like UV-Spectrophotometry[38-40], voltametric polaro-graphic method[41,42], HPLC[43-47], in biological samples, plasm[48] and serum[49]. Two methods have been reported for simultaneous analysis of ATL and LER in its combination which includes TLC densitometry and second order derivative spectrophoto-metry[50,51]. Extensive literature survey reveals that no RP-HPLC method is reported for simultaneous determination of ATL and LER in tablet dosage form. Fixed dose combination containing atenolol (50mg) and Lercanidipine (10mg) is available in tablet form in the market. Therefore, an attempt was made to develop a new, rapid and sensitive RP-HPLC method for the simultaneous determination of ATL and LER in tablet dosage form. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines, which is mandatory also[52,53]. (See figure 1.A and 1.B).

To the best of our knowledge, no study has been reported for the simultaneous determination of Atenolol and Lercanidipine in pharmaceutical formulations by UV-spectrophotometer and RP-HPLC method. The significance of the developed methods is to determine the content of both drugs simultaneously in commercially available capsule dosage form and can be used in future for bioequivalence study for the same formulations. The Tablet solid dosage form in combination containing Atenolol (50 mg) and Lercanidipine (10 mg) is available in the market. In this paper, we reported one reverse-phase stability indicating HPLC method for the quantification of Atenolol and Lercanidipine simultaneously. The present RP-HPLC method was validated as per ICH guidelines[52,53].

However there is no analytical method reported for simultaneous estimation of both drugs in their combined tablet dosage form by reporting forced degradation studies to demonstrate stability indicating nature of the method. Present work describes rapid, simple, sensitive, accurate and reproducible stability indicating method.The present developed method was used determine the Atenolol and Lercanidipine present in the formulation and method validated according to the ICH guidelines[52,53].

MATERIALS AND METHODS

Materials

HPLC grade Potassium dihydrogen phosphate, acetonitrile, methanol and water were procured from Merck India. All dilutions were performed in standard class-A, volumetric glassware. For the estimation of commercial formulation, Lotensyl-AT having (Atenolol-50mg and Lercanidipine-10mg) manufactured by Sun pharma-

ceuticals industries ltd were procured from the local market.

Instrumentation

Waters Alliance HPLC, with DAD detector was used for the chromatographic separation. The chromatogram was recorded at and peaks quantified by means of Empower-2 software. Chromatographic separation was carried out on a C18 column [X-terra RP-18, 150mm x4.6mm 5µ]. Sartorius electronic balance was used for weighing the samples. Ultra-sonic bath sonicator was used for degassing and mixing of the mobile phase.

Chromatographic conditions

Chromatographic separation of Atenolol and Lercanidipine was carried on a C18 column. The mobile phase was composed of acetonitrile, methanol and a mixed buffer of 0.02M potassium dihydrogen phosphate and Potassium Dihydrogen Phosphate buffer (pH 5.0) in the ratio of 50:10:40 v/v. It was filtered through a 0.45 µ membrane filter and degassed for 15 minutes. The flow rate of the mobile phase was maintained at 1 ml/min. Detection was carried out at 226 nm at ambient temperature.

Method development

Preparation of standard stock solutions

Standard stock solutions were prepared by dissolving 52 mg of Atenolol and 20 mg Lercanidipine working standard in two separate each 25mL and 50 mL volumetric flasks using 30mL of mobile phase and made up to the mark with mobile phase to obtain a final concentration of 2000µg/mL and 400 µg/mL of each Atenolol and Lercanidipine. From the above stock solutions, each 5ml of aliquots of Atenolol and Lercanidipine were pipette in to a 25mLvolumetric flask and dissolved in 25mL of the mobile phase and made up to the mark with the solvent to obtain a final concentration of 400 µg/mL and 80 µg/mL for Atenolol and Lercanidipine respectively.

Preparation of sample solutions

Weighed and finely powdered 20 Tablets. Accurately weighed and transferred equivalent to 50mg Atenolol and 10mg of Lercanidipine into a 25 mL volumetric flask, added 20 mL of diluent, and sonicated for 30minutes with intermittent shaking at controlled temperature and diluted to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transferred 5.0 mL of the above solution into a 25 mL volumetric flask and diluted to volume with diluent to obtain a concentration of 400 and 80 µg/mL of Atenolol and Lercanidipine respectively.

Method validation

The developed HPLC method for the simultaneous determination of Atenolol and Lercanidipine was validated as per the ICHguidelines[13,14].

As part of method validation as per ICH guidelines, the following parameters are studied.

1. System Suitability and System Precision
2. Specificity Studies

A. Blank Interference
B. Placebo Interference
C. Forced degradation studies in different stress conditions to establishing stability indication of the developed method.

3. Method Precision
4. Accuracy studies
5. Linearity Studies including LOD/LOQ determination
6. Ruggedness
7. Robustness
8. Analysis of Marketed samples by applying the developed method.

Each parameter was explained separately in different sections under results and discussions.

RESULTS AND DISCUSSION

System suitability and system precision

System suitability for chromatographic separation was checked on each day of validation to evaluate the components of the analytical system in order to show that the performance of the system meet the standards required by the method. System suitability parameters established for the developed method include number of theoretical plates (efficiency), Resolution, Tailing factor. The HPLC system was equilibrated using the initial mobile phase composition, followed by 5 injections of the standard solution of 100% concentration containing 400 µg/mL Atenolol and 80 µg/ml Lercanidipine. These 5 consecutive injections were used to evaluate the system suitability on each day of method validation. The result was given in the Table 1.

Specificity

Blank interference

A study to establish the interference of blank was conducted. Diluent was injected into the chromatograph in the defined above chromatographic conditions and the blank chromatograms were recorded. Chromatogram of Blank solution (Fig. no. 2) showed no peaks at the retention time of Atenolol and Lercanidipine peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Atenolol and Lercanidipine in Atenolol and Lercanidipine tablets. Similarly typical representative chromatogram of standard is also shown (Fig. no. 3).

Forced degradation

Control sample

Weighed and finely powdered 20 Tablets. Accurately weighed and transferred equivalent to 50mg Atenolol and 10mg of Lercanidipine into a 25 mL volumetric flask, added 20 mL of diluent, and sonicated for 30minutes with intermittent shaking at controlled temperature and diluted to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transferred 5.0 mL of the above solution into a 25 mL volumetric flask and diluted to volume with diluent. Refer **(**Fig. no. 4A**).**

Acid degradation sample

Weighed and finely powdered 20 Tablets. Accurately weighed and transferred equivalent to 50mg Atenolol and 10mg of Lercanidipine into a 25 mL volumetric flask, added 15 mL of diluent, and sonicated for 30minutes with intermittent shaking at controlled temperature. Then add 2.0mL of 1N acid, refluxed for 30min at 60°C, then cooled to room temperature, neutralize with 1N NaOH and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 2.0 mL of the above solution into a 25 mL volumetric flask and dilute to volume with diluent. Refer (Fig. no. 4B)

Base degradation sample

Weighed and finely powdered 20 Tablets. Accurately weighed and transferred equivalent to 50mg Atenolol and 10mg of Lercanidipine into a 25 mL volumetric flask, added 15 mL of diluent, and sonicated for 30minutes with intermittent shaking at controlled temperature. Then add 2.0mL of 1N NaOH, refluxed for 30min at 60°C, then cooled to room temperature, neutralize with 1N HCl and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 2.0 mL of the above solution into a 25 mL volumetric flask and dilute to volume with diluent. Refer (Fig. no. 4C).

Peroxide degradation sample

Weighed and finely powdered 20 Tablets. Accurately weighed and transferred

equivalent to 50mg Atenolol and 10mg of Lercanidipine into a 25 mL volumetric flask, added 15 mL of diluent, and sonicated for 30minutes with intermittent shaking at controlled temperature. Then add 2mL of Hydrogen Peroxide, refluxed for 10min at 60°C, then cooled to room temperature, and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 5.0 mL of the above solution into a 25 mL volumetric flask and dilute to volume with diluent. Refer (Fig. no. 4D).

Thermal degradation sample

Tablets are exposed to 105°c for five days. Weighed and finely powdered 20 Tablets. Weighed and finely powdered 20 Tablets. Accurately weighed and transferred equivalent to 50mg Atenolol and 10mg of Lercanidipine into a 25 mL volumetric flask, added 15 mL of diluent, and sonicated for 30minutes with intermittent shaking at controlled temperature. And dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 5.0 mL of the above solution into a 100 mL volumetric flask and dilute to volume with diluent. Refer (Fig. no. 4E).

Similarly Humidity, UV-Light exposure, Sunlight exposure and Water hydrolysis stress samples are prepared and checked for their purity by proposed method.

Linearity and range

The standard curve was obtained in the concentration range of 208-624 µg/ml for Atenolol and 40-120 µg/mL for Lercanidipine. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r2] of standard curve were calculated and given in Figure-5A (For Atenolol) and Figure-5B (For Lercanidipine) to demonstrate the linearity of the proposed method. The result of regression analysis was given in the Table 2.

From the data obtained which given in Table-2 (For Atenolol and Lercanidipine) the method was found to be linear within the proposed range.

Accuracy

The accuracy of an analytical method is the closeness of results obtained by that method to the true value for the sample. It is expressed as recovery (%), which is determined by the standard addition method. In the current study recovery at three spike levels 50%, 100% and 150% were carried out. The % recovery at each spike level was calculated and was given in Table 3.

Precision

The precision of an analytical method is the closeness of replicate results obtained from analysis of the same homogeneous sample. Precision was considered at different levels, i.e. method, system, Inter day and intraday. Precision of the developed method was assessed by measuring the response on the same day (intraday precision) and next two consecutive days (inter day precision). The precision of the method was assessed by six replicate injections of 100% test concentration. Intra and inter-day precision of the method was assessed by determination of standard deviation and % RSD for the analyte response. The result was given in Table 4.

LOD and LOQ

LOD and LOQ values were determined by the formulae LOD = 3.3 σ/S and LOQ = 10 σ/S (Where, σ is the standard deviation of the responses and S is the slope of the calibration curves). In the present method σ is the mean of standard deviation of y intercepts of the three calibration curves and S is the mean of slopes of the calibration curves. The result was given in Table5.

Robustness

The robustness of the method was determined by assessing the ability of the developed method to remain unaffected by the small changes in the parameters such as percent organic content, pH of the mobile phase, buffer concentration, temperature, injection volume and flow rate. A deviation of ± 2nm in the detection wavelength, ± 0.1 mL/min in the flow rate, ± 5%change in the organic phase were tried individually. The result was given in the Table 5.

OVER ALL SUMMARY OF THE METHOD

Column chemistry, solvent selectivity, solvent strength (volume fraction of organic solvent (s) in the mobile phase), detection wavelength and flow rate were varied to determine the chromatographic conditions for giving the best separation. Several mobile phase compositions were tried to resolve the peaks of Atenolol and Lercanidipine. The optimum results were attained with acetonitrile, methanol and potassium dihydrogen phosphate buffer (pH 5.0) in the ratio of 50:10:40 (v/v) because it could resolve the peaks of Atenolol with retention time at 2.0 min and Lercanidipine retention time at 3.7 min. The two peaks were symmetric and sufficiently resolved. System suitability was carried out by injecting 5 replicate injections of 100% concentration of Atenolol and Lercanidipine. The resolution was found to be greater than 2 and the other parameters are presented in Table 1.

Specificity of the chromatographic method was tested by injecting mobile phase as blank and sample concentration prepared from marketed formulation. The response was compared with that obtained from the standard drug. The chromatogram confirms the presence of Atenolol and Lercanidipine at 2.0 min and 3.7 min respectively without any interference. Thus the developed method was specific for analyzing the commercial formulations for Atenolol and Lercanidipine. An optimized chromatogram with the retention times of Atenolol and Lercanidipine was shown in the Figure 2.

The peak areas corresponding to the concentration range of Atenolol 208-624 µg/mL and Lercanidipine 40-120 µg/ml prepared in triplicate were plotted against the respective concentrations. The calibration curves were linear in the range studied for Atenolol and Lercanidipine, respectively, with mean correlation coefficients (n=3) of 0.999 and higher, the representative calibration curve is shown in Figure 3. The regression analysis was given in Table 2.

Accuracy of the proposed method was assessed by standard addition method at 50%, 100% and 150% levels of recovery to the pre analyzed sample in triplicate. The recovery of the added standard to the sample was calculated and it was found to be 97-101 %w/w for Atenolol and 98-101 %w/w for Lercanidipine respectively and the % RSD was less than 2 for both the drugs which indicates good accuracy of the method. The result of recovery was given in table 3.

LOD and LOQ were calculated from the average slope and standard deviation of y intercepts of the calibration curve. Limit of detection for Atenolol and Lercanidipine were 16.50µg/mL and 7.43µg/mL respectively where as limit of quantitation of Atenolol and Lercanidipine were 50.00 µg/mL and 22.50 µg/mL respectively indicating high sensitivity of the method. LOD and LOQ value was given in table 2. The method is precise with a % RSD of less than 2 for both Atenolol and Lercanidipine respectively. The results of intraday and inter day precision was given in table 4. Robustness was carried out by change in the flow rate (±1mL/min), mobile phase variation (±5%) and variation in wavelength (± 2 nm).Solution of 100% concentration is prepared and injected in triplicate for each varied operational condition and % R.S.D was found to be less than 2. The result was

given in table 5. The proposed method was applied for the assay of commercial formulation containing Atenolol and Lercanidipine. Each sample was analyzed in triplicate. The mean recovery values were 98.5 and 99.4 for Atenolol and Lercanidipine. The result of estimation was given in table 6.

CONCLUSION

The proposed RP-HPLC method for simultaneous assay Atenolol and Lercanidipine in combined dosage forms was validated, and found to be applicable for routine quantitative analysis of Atenolol and Lercanidipine. The results of linearity, precision, accuracy and specificity, were proved to be within the limits. The method provides selective quantification of Atenolol and Lercanidipine with no interference from other formulation excipients. Therefore, this method can be employed for the routine analysis for simultaneous estimation Atenolol and Lercanidipine in quality control of formulations and also in the dissolution studies.

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Table 1. System suitability parameters for atenolol and Lercanidipine by proposed method

Name of the compound	Retention time	Tailing factor	Theoretical plate	USP resolution
Atenolol	2.072	1.522	3693	-
Lercanidipine	3.755	1.623	5575	9.400

Table 2. Linearity studies for atenolol and Lercanidipine by proposed method

% Level (approx.)	For atenolol		For lercanidipine	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
50	208	1936952	39.6	964943
75	312	2905389	59.4	1447410
100	416	3873864	79.2	1929868
125	520	4842330	99	2412335
150	624	5810815	118.8	2894820
	Slope	9312	Slope	24367
	Intercept	3.0	Intercept	4.0
	% Y-Intercept	0.0	% Y-Intercept	0.0
	STYEX	15.0	STYEX	9.0
	CC	1.0000	CC	1.0000
	RSQ	1.0000	RSQ	1.0000
	Residual sum of squares	15.0	Residual sum of squares	9
	LLD	16.50	LLD	7.43
	LLQ	50.00	LLQ	22.50

Table 3A. Recovery studies for atenolol by proposed method

% Level	Recovery range	% RSD at each level	Over all %RSD
50	98.02-99.44	0.44	0.44
100	99.17-99.49	0.17	
150	100.49-101.10	1.20	

Table 3B. Recovery studies for Lercanidipine by proposed method

% Level	Recovery range	% RSD at each level	Over all %RSD
50	98.83-100.22	0.79	0.31
100	97.57-100.41	1.45	
150	98.73-100.21	0.76	

Table 4. Method precision (Inter and Intraday) studies for atenolol and Lercanidipine by proposed method

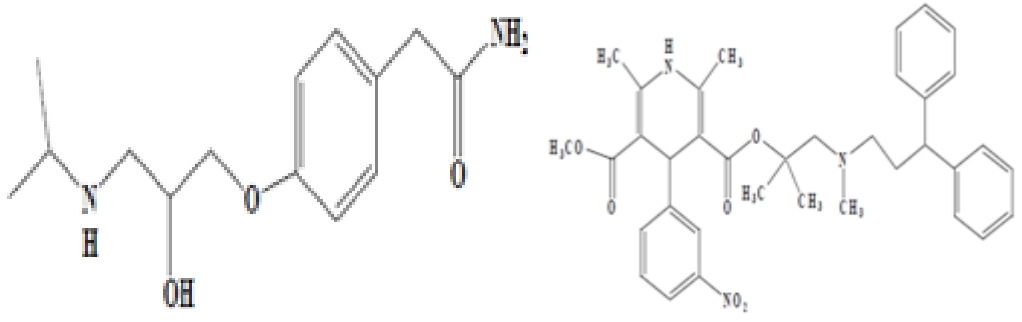
Summary showing method Precision by Proposed method			
For atenolol		For lercanidipine	
Method precision (Inter &Intra Day)		Method precision (Inter &Intra Day)	
100.22	99.44	99.81	100.22
99.86	99.90	98.19	100.16
97.85	99.02	101.46	98.83
98.95	100.30	99.63	99.13
97.71	100.60	101.27	98.83
98.59	97.91	99.02	101.08
Overall Avg.	99.20		99.82
Overage Std Dev.	1.02		1.05
Over all %RSD	1.02		1.05

Table 5. Robustness studies for atenolol and Lercanidipine by proposed method

Parameter		% RSD	
		Atenolol	Lercanidipine
Wavelength ±2	224 nm	0.42	0.54
	228 nm	0.54	0.47
Flow rate mL /min	0.8 mL/min	0.12	0.34
	1.2mL.min	0.49	0.52

Table 6. Assay of marketed samples for atenolol and Lercanidipine by proposed method

Drug	Amount claimed in mg per Tablet	Estimated amount in mg/tablet	% Assay
Atenolol	50	48.85	97.7
Lercanidipine	10	9.88	98.8



(A) (B)

Figure 1. A- Atenolol, B- Lercanidipine

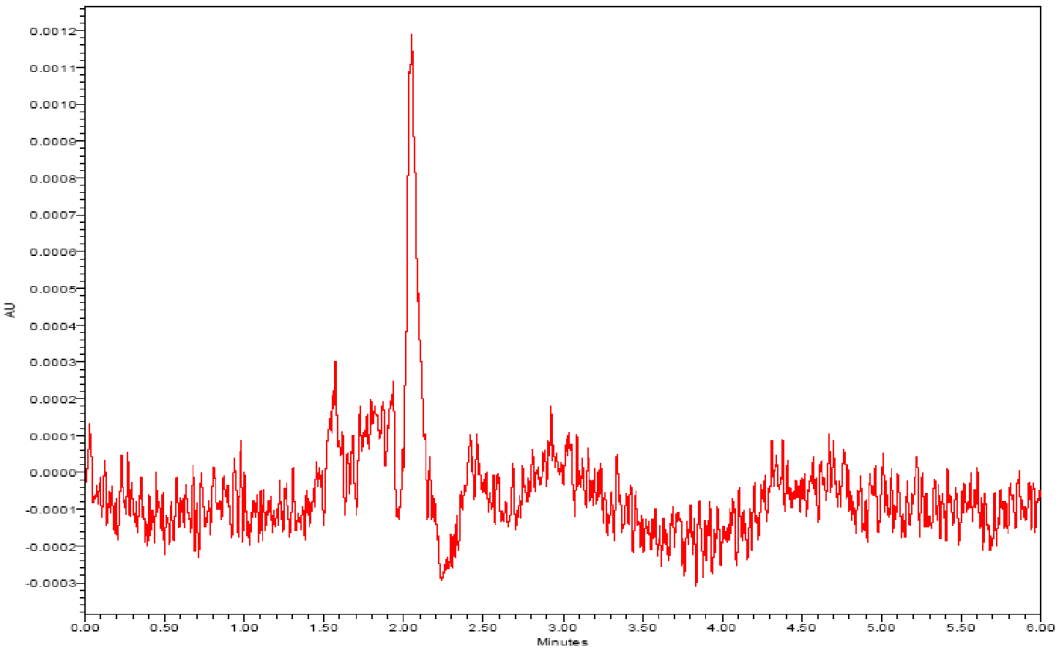


Figure 2. A typical HPLC Chromatogram showing the no interference of diluent for Atenolol and Lercanidipine

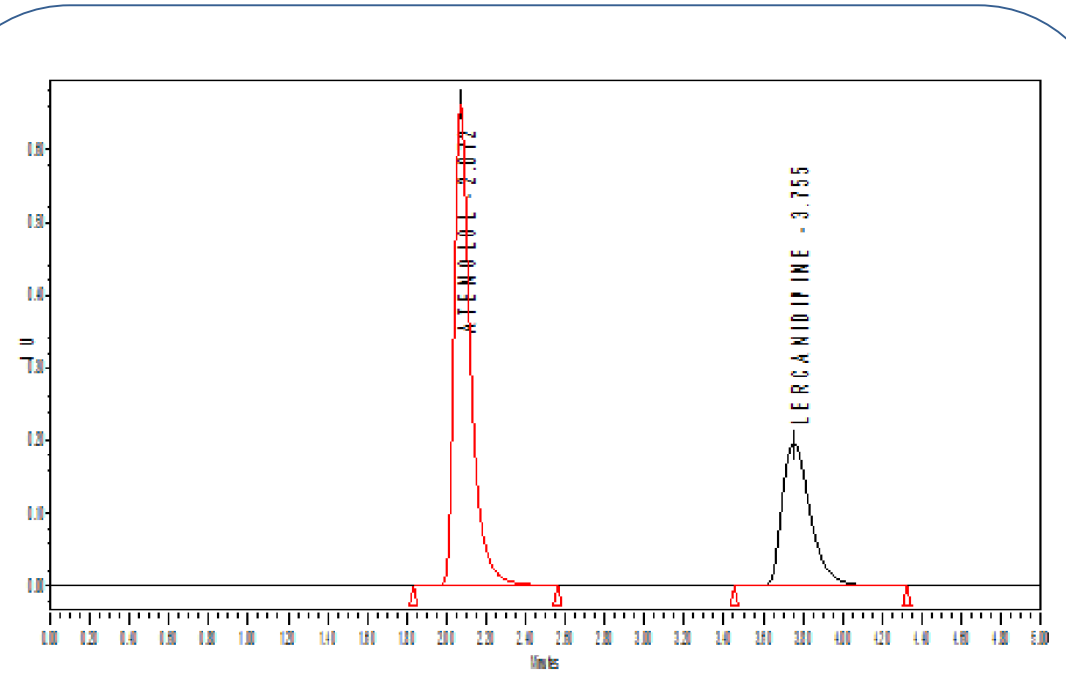


Figure 3. A typical HPLC Chromatogram showing the peak of Atenolol and Lercanidipine

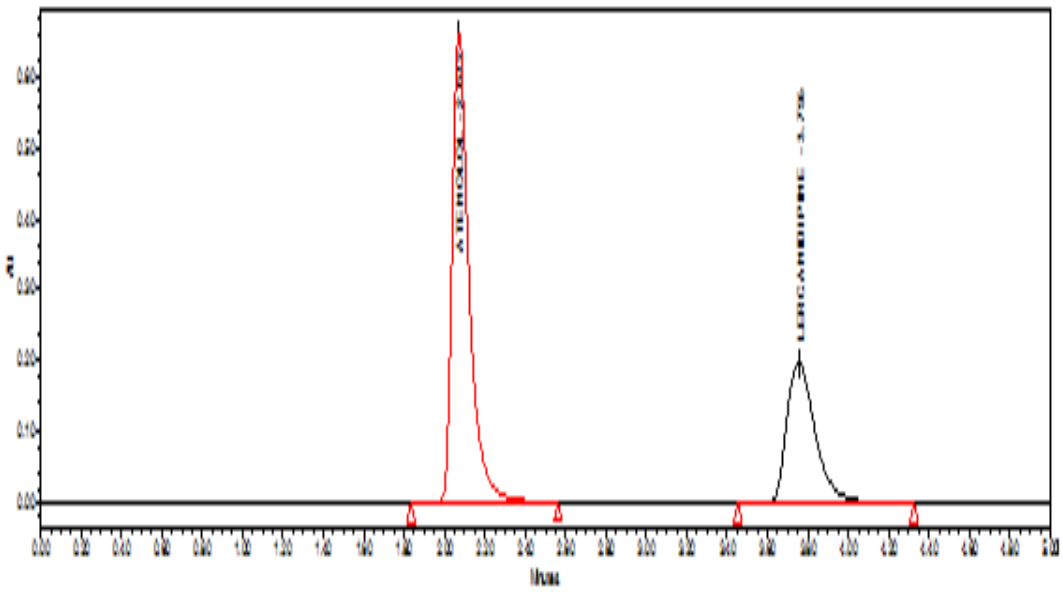


Figure 4A. A typical HPLC Chromatogram showing the Control Sample profile of Atenolol and Lercanidipine by proposed method

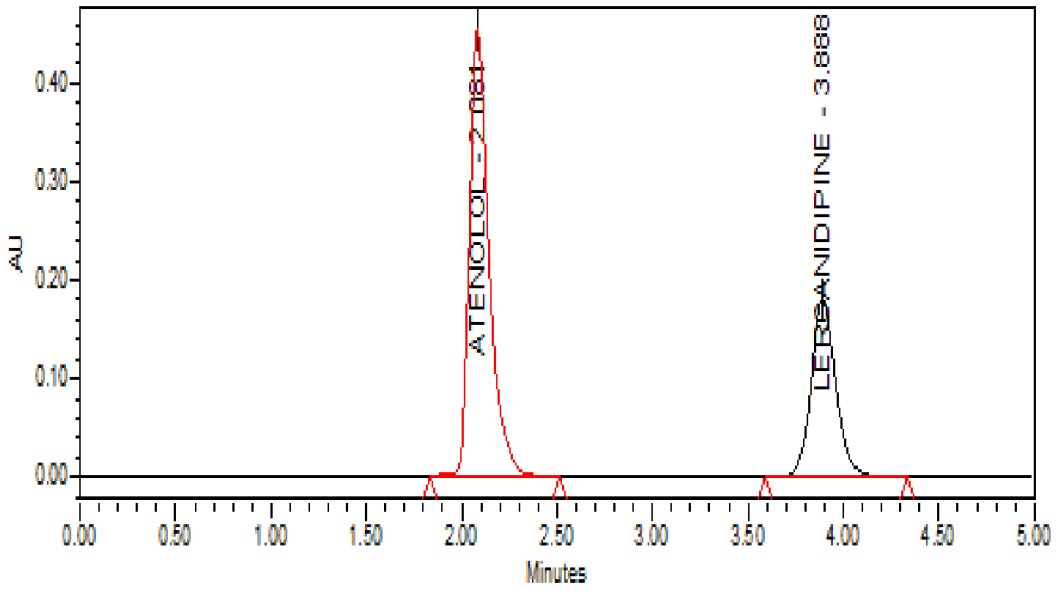


Figure 4B. A typical HPLC Chromatogram showing the profile of Atenolol and Lercanidipine in Acidic hydrolysis by proposed method

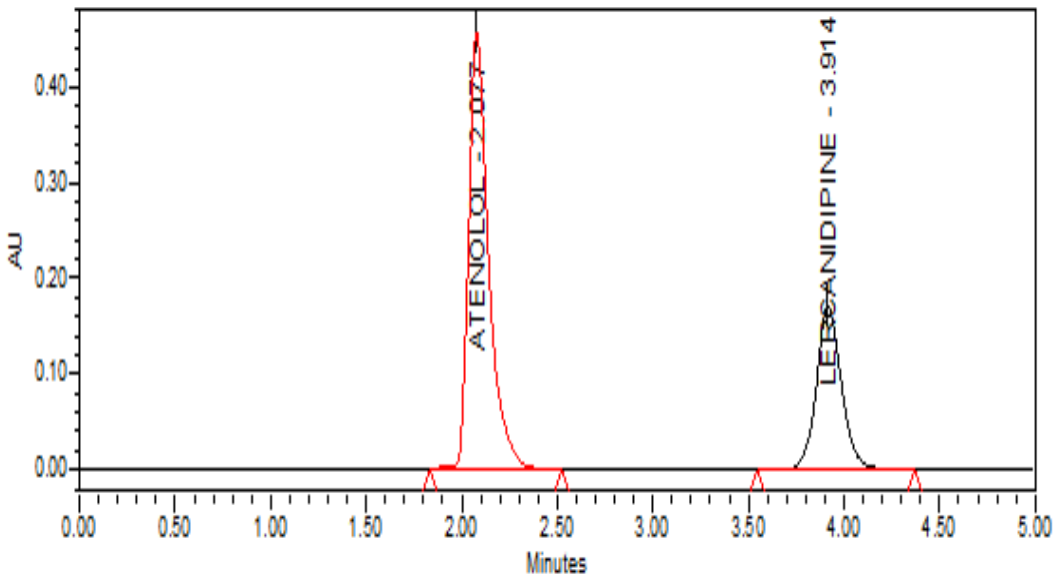


Figure 4C. A typical HPLC Chromatogram showing the profile of Atenolol and Lercanidipine in Base hydrolysis by proposed method

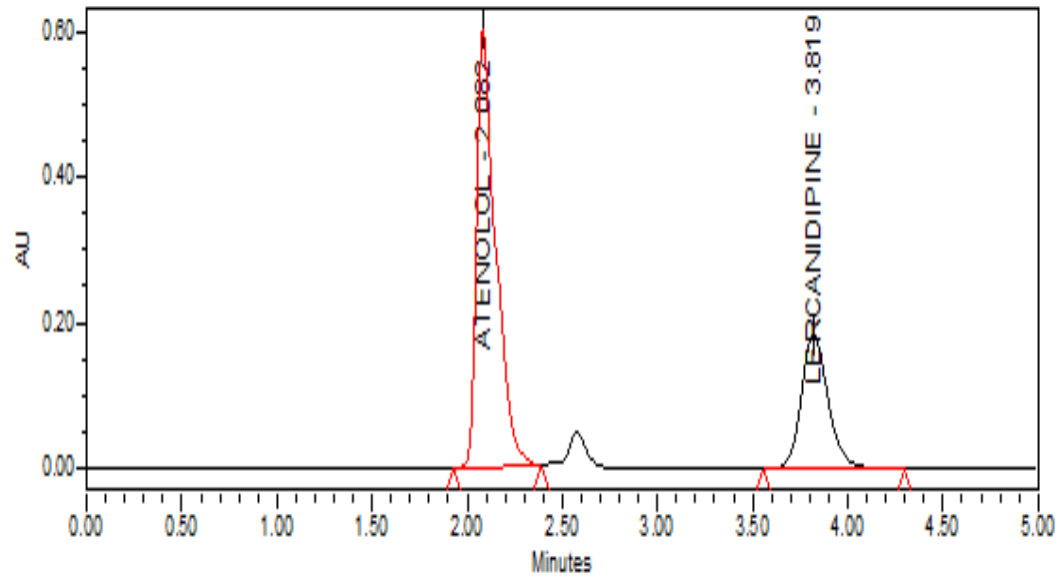


Figure 4D. A typical HPLC Chromatogram showing the profile of Atenolol and Lercanidipine in Peroxide hydrolysis by proposed method

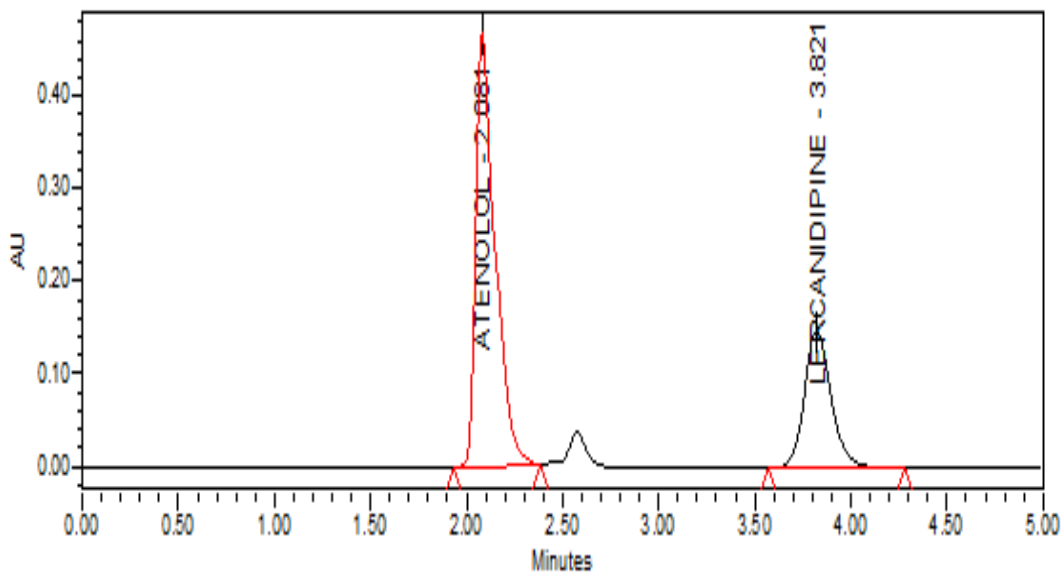


Figure 4E. A typical HPLC Chromatogram showing the profile of Atenolol and Lercanidipine in Thermal hydrolysis by proposed method

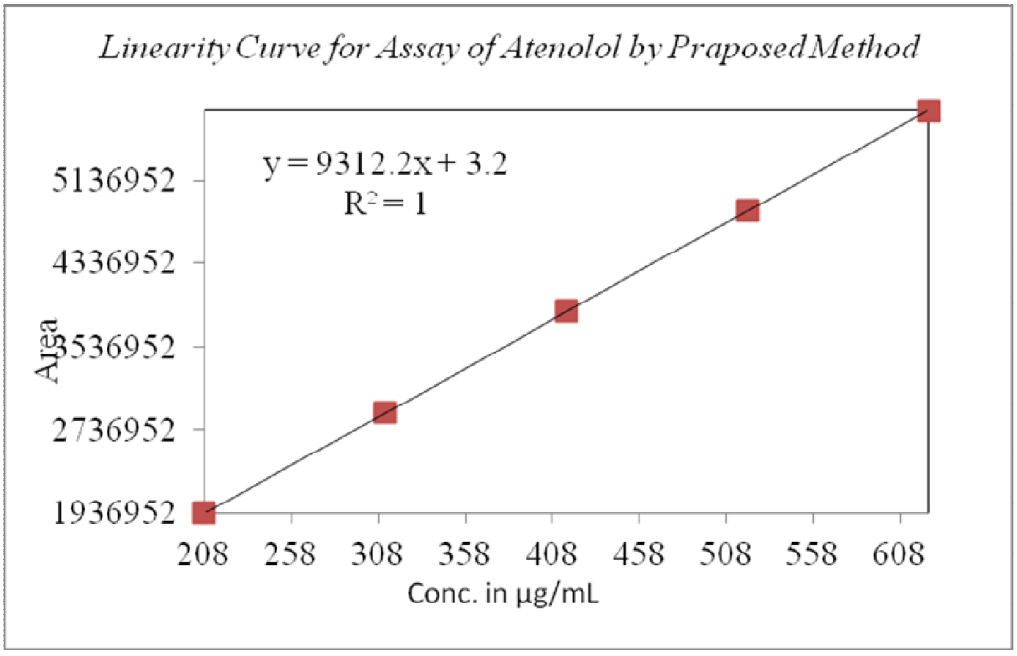


Figure 5A. Calibration curve for atenolol

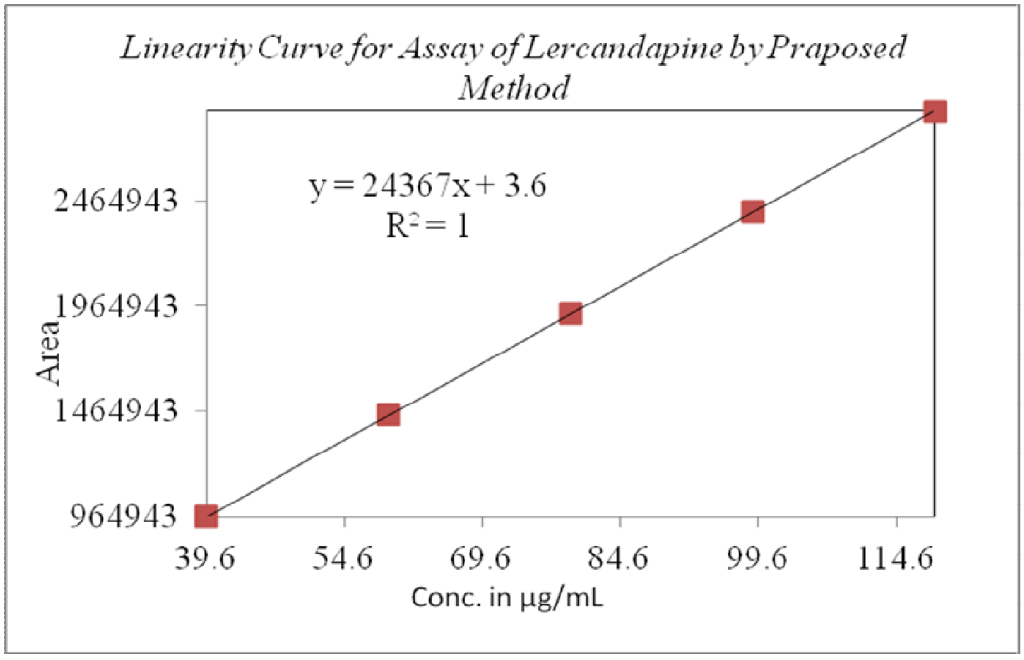


Figure 5B. Calibration curve for Lercanidipine