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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF ATENOLOL AND LERCANDIPINE IN A PHARMACEUTICAL FORMULATION BY RP-HPLC METHOD

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ABSTRACT

An isocratic Simultaneous estimation by RP-HPLC Method were developed and validated for the quantification of Atenolol and Lercandipine in tablet dosage form. Quantification was achieved by using a reversed-phase C18 column (INERTSIL 3V ODS Column, 5µ, 250 mm × 4.6 mm) at ambient temperature with mobile phase consisting of ammonium acetate: THF: Acn(35:10:55)_pH-3. The flow rate was 1.0 ml/min. Measurements were made at a wavelength of 221nm. The average retention time for Atenolol and Lercandipine were found to be 2.55 min and 4.410. The proposed method was validated for selectivity, precision, linearity and accuracy. The assay methods were found to be linear from 60-140µg/ml for Atenolol and 12 to 28µg/ml for Lercandipine. All validation parameters were within the acceptable range. The developed methods were successfully applied to estimate the amount of Atenolol and Lercandipine in tablet dosage form.

KEYWORDS

Atenolol, Lercandipine, RP-HPLC method, INERTSIL 3V ODS, THF, Acetonitrile, Ammonium Acetate, Ortho phosphoric acid and Validation.

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INTRODUCTION

Atenolol (Figure No.1) is a selective β_1 receptor antagonist, a drug belonging to the group of beta blockers, a class of drugs used primarily in cardiovascular diseases. Introduced in 1976, atenolol was developed as a replacement for propranolol in the treatment of hypertension. The chemical works by slowing down the heart and reducing its workload. Unlike propranolol, atenolol does not pass

through the blood–brain barrier thus avoiding various central nervous system side effects. The beta-1 adrenergic receptor (β1 adrenoreceptor), also known as ADRB1, is a beta-adrenergic receptor, and also denotes the human gene encoding it[1]. It is a G-protein coupled receptor associated with the Gs heterotrimeric G-protein and is expressed predominantly in cardiac tissue.

Medical uses: Atenolol is used for a number of conditions including: hypertension, angina, acute myocardial infarction, supraventricular tachycardia, ventricular tachycardia, and the symptoms of alcohol withdrawal.

Side Effects

Indigestion, constipation, dry mouth, dizziness or faintness (especially cases of orthostatic hypotension), cold extremities, impotence, rhinitis.

Lercanidipine (Figure No.1) is a calcium channel blocker of the dihydropyridine class, which works by relaxing and opening the blood vessels allowing the blood to circulate more freely around the body. It is sold under various commercial names including Zanidip. This lowers the blood pressure and allows the heart to work more efficiently.

A calcium channel blocker (CCB) is a chemical that disrupts the movement of calcium (Ca^{2+}) through calcium channels[1]. Calcium channel blockers are used as antihypertensive drugs, i.e. as medications to decrease blood pressure in patients with hypertension. CCBs are particularly effective against large vessel stiffness, one of the common causes of elevated systolic blood pressure in elderly patients[2]. Calcium channel blockers are also frequently used to alter heart rate, to prevent cerebral vasospasm, and to reduce chest pain caused by angina pectoris. One type of calcium channel blocker is used experimentally to prevent migraine, and another one is used as a powerful painkiller.

Side Effects

Drowsiness, sweating, dry mouth and headache[1-5].

MATERIAL AND METHOD

Instruments the chromatographic technique performed on a Shimadzu LC20-AT Liquid chromatography with SPD-20A prominence UV-visible detector and Spinchrom software, reversed phase C18 column. INERTSIL 3V Electron Corporation double beam UV-visible spectrophotometer (vision pro-software), Ultrasonic cleaner, Shimadzu analytical balance AY-220, Vaccum micro filtration unit with 0.45µ membrane filter was used in the study.

Materials

Pharmaceutically pure sample of Atenolol and Lercandipine were obtained as gift samples from Chandra laboratories pvt ltd, Prashanthinagar, Kukatpally, Hyderabad, India. The purity of the drug was evaluated by obtaining its melting point and ultraviolet (UV) and infrared (IR) spectra. No impurities were found. The drug was used without further purification.

HPLC-grade Acetonitrile and THF ware from standard reagents pvt ltd. Ammonium acetate (AR grade) was from Merck.

A tablet formulation of Atenolol and Lercandipine (50 mg and 10mg label claims) were procured from local market (LOTENSYL-AT, Sun Pharmaceutical Industries Ltd, India).

Determination of working Wavelength (λmax)[6-8]

10 mg of the Atenolol standard drug is taken in a 10 ml volumetric flask and dissolved in methanol and volume made up to the mark, from this solution 0.1ml is pipetted into 10 ml volumetric flask and made upto the mark with the methanol to give a concentration of 10 µg/ml. The above prepared solution is scanned in UV between 200-400 nm using methanol as blank. The λmax was found to be 207nm (Figure No.3).

10 mg of the Lercandipine standard drug is taken in a 10 ml volumetric flask and dissolved in methanol and volume made up to the mark, from this solution 0.1ml is pipetted into 10 ml volumetric flask and made upto the mark with the methanol to give a concentration of 10 µg/ml. The above prepared solution is scanned in uv between 200-400 nm using methanol as blank. The λmax was found to be 216nm (Figure No.4).

The Iso bestic Point of Atenolol and Lercandipine were found to be 221nm (Figure No.5).

Preparation of mobile phase

Buffer Preparation

Weigh accurately about 3.85 gms of Ammonium acetate and dissolve with 200ml of HPLC Grade water than make up to 1000 ml with HPLC grade water then adjust the pH: 3.0 with ortho phosphoric acid.

Mobile phase

Then add 35 volumes of buffer, 55 volumes of Acetonitrile and 10volumes of THF and sonicated for 15 min and filtered through a 0.45 µ membrane filter.

Analysis of formulation

Preparation of standard solution

A 50mg of standard Atenolol and 10 mg Lercandipine ware weighed and transferred to 50 ml of volumetric flask and dissolved in mobile phase. The flask was shaken and volume was made up to mark with mobile phase to give a primary stock solution containing 1000µg/ml Atenolol and 200µg/ml of Lercandipine. From the above solution 5ml of solution is pipetted out into a 50 ml volumetric flask and volume was made up to mark with mobile phase to give a solution containing 100µg/ml Atenolol and 20µg/ml of Lercandipine.

Preparation of sample solution

For the estimation of the drug in tablet formulation twenty tablets were weighed and their average weight was determined. The tablets were then finely powdered. Appropriate quantity equivalent to 50mg Atenolol and 10 mg Lercandipine ware accurately weighed and The powder was transferred to 50 ml volumetric flask and shaken vigorously with mobile phase and sonicated for 15 min and volume made up to the mark with mobile phase. The solution was shaken vigorously and filtered by using whatmann filter no.41. from the above filtered clear solution 5ml of sample pipetted out into a 50 ml volumetric flask volume made up to the mark with mobile phase to give a solution containing 100µg/ml Atenolol and 20µg/ml of Lercandipine.

Calculation 5 replicates of each of sample and standard solutions were injected and their average peak areas were taken. The amount of Atenolol and Lercandipine present in the formulation by using the formula given below, and results shown in above Table No.6.

$$\%\ \text{Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where,

AS: Average peak area due to standard preparation
AT: Peak area due to assay preparation
WS: Weight of standard drug taken
WT: Weight of sample in assay preparation
DT: Dilution of assay preparation
DS: Dilution of standard preparation
AW: Average weight of 20 tablets
LC: Label claim
P: Purity of standard drug.

METHOD VALIDATION

Linearity

Linearity was studied by analyzing five standard solutions covering the range of 60-140µg/ml for Atenolol and 12-28 µg/ml for Lercandipine of the drug. From the primary stock solution 0.6ml, 0.8ml, 1.0ml, 1.2ml and 1.4 ml of aliquots are pipetted into 10 ml volumetric flasks and made up to the mark with the mobile phase to give a concentrations of 60µg/mL, 80µg/mL, 100µg/mL, 120µg/mL and 140 µg/mL of Atenolol and 12µg/mL, 16µg/Ml, 20µg/mL, 24µg/mL, 28µg/mL of Lercandipine.

Calibration curve (Figure No.2 and Table No.1) with concentration verses peak areas was plotted by injecting the above prepared solutions and the obtained data were subjected to regression analysis using the least squares method.

Method precision (repeatability)

The precision of the instrument was checked by repeated injections and measurement of peak areas and retention times of solutions (n = 6) for, 100µg/ml of Atenolol and 20µg/ml of Lercandipine without changing the parameter of the proposed chromatographic method.

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 different days over a period of 1 week for 100µg/ml and 20µg/ml concentrations of standard solutions of Atenolol and

Lercandipine. The result was reported in terms of relative standard deviation (% RSD).

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) (Table No.2) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (1) and (2), respectively.

$$\mathbf{LOD = 3.3\ \delta/S \ldots\ldots\ldots (1)}$$

$$\mathbf{LOQ = 10\ \delta/S \ldots\ldots\ldots (2)}$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of Atenolol and Lercandipine by the standard addition method. Known amounts of standard solutions of Atenolol and Lercandipine were added at 20% concentration to pre quantified sample solutions of Atenolol (100,120, 140µg/ml) and Lercandipine (20, 24, 28µg/ml) (Figure No.7 and 8 and Table No.3). The amount of Atenolol and Lercandipine recovered was estimated by using the following formulas.

$$\%\ Recovery = \frac{amount\ found}{Amount\ added} \times 100$$

$$Amount\ Found(mcg/ml) = \frac{Mean\ test\ area}{Mean\ standard\ area} \times Standard\ concentration$$

Specificity

In an assay (Table No.6), demonstration of specificity requires that it can be shown that the procedure is unaffected by the presence of impurities or excipients. In practice, this can be done by spiking the drug substance or product with appropriate levels of impurities or excipients and demonstrating that the assay results are unaffected by the presence of these extraneous materials. There should be no interference of the diluents, placebo at retention time of drug substances (Figure No.9).

Robustness

Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied ±2nm and flow rate was varied ±0.2 ml/min. The results were shown in Table No.4 (1 and 2).

Ruggedness

The ruggedness of the method was studied by analyzing the sample and standard preparations by two analysts. The % RSD assay values between two analysts was calculated i.e., (limit <2%). This indicates the method was rugged. The results were shown in Table No.5.

RESULTS AND DISCUSSION

In RP HPLC method, the primary requirement for developing a method for analysis is that the using different solvents and buffers and columns to get better retention time and theoretical plates, and better cost effective and time saving method than the previously developed methods. The Iso bestic Point of Atenolol and Lercandipine were found to be 221nm (Figure No.5) by scanning in UV region. The chromatographic method was optimised with mobile phase consisting of Ammonium acetate Buffer: Acetonitrile: THF (35: 55:10) and C18 INERTSIL 3V ODS column. All the validation parameters were studied at a the wavelength 221nm. Accuracy was determined by calculating the recovery (Table No.3) and the results were in acceptable range (limit 98-102%). The method was successfully used to determine the amount of Atenolol and Lercandipine present in the Tablet. The results obtained were in good agreement with the corresponding labeled amount. The method was linear in the concentration range of 60 to 120µg/ml for Atenolol and 12 to 28µg/ml for Lercandipine (Figure No.6, Table No.1). Precision was calculated as repeatability and intra and inter day variations (% RSD) for the drug (Table No.7 and 8). Robustness and ruggedness results were in acceptable range (Table No.4 and Table No.5). Summary of all validation parameters for method is given in Table No.9. By observing the validation parameters, the method was found to be simple, sensitive, accurate and precise. Hence the method can be employed for the routine analysis Atenolol and Lercandipine in tablet dosage form.

Table No.1: Linearity

S.No	Atenolol		Lercandipine Hcl	
	Mcg	Area	mcg	Area
1	60	1344.606	12	138.783
2	80	1829.853	16	168.306
3	100	2208.421	20	200.849
4	120	2673.186	24	232.682
5	140	3106.591	28	263.873

Table No.2: LOD and LOQ values from calibration curve

S.No	Atenolol		Lercandipine Hcl		
	Mcg	Area	mcg		Area
1	60	1344.606	12		138.783
2	80	1829.853	16		168.306
3	100	2208.421	20		200.849
4	120	2673.186	24		232.682
5	140	3106.591	28		263.873
Std dev	31.6	691	**Std dev**	6.325	50
Slope	21.83		**Slope**	7.683	

S.No		Atenolol		Lercandipine Hcl	
		mcg	Area	mcg	Area
1	**LOD**	4.783	104.44	2.65	20.88
2	**LOQ**	14.49	316.49	38.04	63.26

Table No.3: Recovery data

S.No	Level	Amount of Sample taken (%)	Amount of Standard Spiked (%)	% Recovery of Atenolol	% Recovery of Lercandipine Hcl
1	I	80	20%	98.32%	100.66%
		80	20%		
		80	20%		
2	II	100	20%	98.40%	98.61%
		100	20%		
		100	20%		
3	III	120	20%	99.71%	99.00%
		120	20%		
		120	20%		

Table No.4 (1): Results of Robustness study

S.No	Parameter	Rt of Atenolol	Tailing factor	Peak Area	% Assay
1	Flow Rate(0.8ml)	3.130	1.258	2467.584	98.21%
2	1.2ml	2.090	1.036	2273.325	
3	1.0ml	2.520	1.308	2344.463	
4	Wave Length 219nm	2.513	1.222	2284.209	99.21
5	223nm	2.517	1.179	2254.896	
6	221nm	2.553	1.467	2281.202	

Table No.4 (2): Results of Robustness study

S.No	Parameter	Rt of Lercandipine	Tailing factor	Peak Area	% Assay
1	Flow Rate(0.8ml)	5.443	1.167	223.965	98.53%
2	1.2ml	3.663	1.012	202.496	
3	1.0ml	4.390	1.152	212.684	
4	Wave Length219nm	4.382	1.088	236.634	99.71%
5	223nm	4.380	1.125	224.802	
6	221nm	4.381	1.125	214.802	

Table No.5: Results of Ruggedness

S.No			Std Area	Spl Area	% Assay	% RSD
1	Analyst-1	Atenolol	2319.879	2305.220	98.02	0.12%
2	Analyst-2		2327.917	2311.653	100.72%	
3	Analyst-1	Lercandipine	208.704	207.958	100.98	1.65%
4	Analyst-2		205.718	207.445	98.19%	

Table No.6: Assay Results

S.No	Atenolol			Lercandipine Hcl		
1	Standard Area	1	2334.362	--	207.967	
		2	2323.199		199.698	
		3	2337.863		207.039	
		4	2331.502		207.632	
		5	2328.483		198.197	
		Average	2331.082	Average	204.1066	
2	Sample area	1	2344.463	--	212.684	
		2	2351.614		209.655	
		3	2337.863		207.039	
		4	2334.732		210.092	
		5	2341.801		210.08	
		Average	2331.082	Average	209.91	
3	Tablet average weight	--	250.2	Mg	250.2	mg
4	Standard weight		50.1	Mg	10.01	mg
5	Sample weight		250.2	Mg	250.2	mg
6	Label amount		50	Mg	10	mg
7	std.purity		99.8	%	98.2	%
8	Cal.:		50.00	mg	10.11	mg
% Assay			100.00	%	101.09	%

Table No.7: Method Precision (Repeatability)

S.No	Atenolol		Lercandipine	
	Rt	Area	Rt	Area
1	2.51	2292.147	4.397	201.545
2	2.523	2322.573	4.410	201.442
3	2.523	2321.138	4.413	202.102
4	2.523	2333.196	4.413	200.853
5	2.507	2350.119	4.397	202.888
6	2.497	2341.355	4.390	198.551
average	2.5138	2326.755	4.403	201.230
St.dev	0.0109	20.235	0.010	1.481
% RSD	0.43	0.87	0.22	0.74

Table No.8: Intermediate Precision

Method Precision (day1):

S.No	Atenolol		Lercandipine Hcl	
	Rt	Area	Rt	Area
1	2.52	2347.345	4.403	212.487
2	2.483	2358.471	4.350	208.188
3	2.520	2350.065	4.403	208.349
4	2.517	2342.378	4.386	217.623
5	2.517	2341.424	4.387	207.526
6	2.523	2356.288	4.417	208.812
Avg	2.5133	2349.329	4.391	210.498
Stdev	0.0150	7.033	0.023	3.908
% RSD	0.60	0.30	0.53	1.86

Method Precision (day2):

S.No	Atenolol		Lercandipine Hcl	
	Rt	Area	Rt	Area
1	2.52	2349.542	4.410	213.497
2	2.522	2346.194	4.401	207.847
3	2.533	2347.022	4.437	214.218
4	2.524	2340.833	4.402	210.656
5	2.503	2350.231	4.394	212.056
6	2.506	2351.204	4.394	208.915
Avg	2.5180	2347.504	4.406	211.198
Stdev	0.0114	3.788	0.016	2.525
% RSD	0.45	0.16	0.37	1.20

Method Precision (day3):

S.No	Atenolol		Lercandipine Hcl	
	Rt	Area	Rt	Area
1	2.511	2349.363	4.414	208.434
2	2.523	2351.355	4.417	214.67
3	2.503	2354.149	4.407	215.149
4	2.512	2348.209	4.340	216.507
5	2.451	2352.633	4.370	205.849
6	2.514	2341.767	4.374	204.207
Avg	2.5023	2349.579	4.387	210.803
Stdev	0.0260	4.389	0.031	5.292
% RSD	1.04	0.19	0.70	1.96

Table No.9: Validation parameters of evaluated method

S.No	Parameter	Value Obtained of Atenolol	Value Obtained Lercandipine
1	Accuracy (% Recovery)	98.12-99.71%	98.61-100.66%
2	Linearity concentrations Range (µ g/mL) Regression coefficient (R2 value)	60-140 µ g/mL 0.999	12-28 µ g/mL 0.998
3	LOD	4.78	2.65
4	LOQ	14.49	8.04
5	Precision (% RSD) Method precision (Repeatability) (% RSD, n = 6)	0.43-0.87	0.22-0.74
6	Intermediate Precision	0.16-1.86	0.37-1.96
7	Robustness (%assay)	98.21-99.21%	98.53-99.71%
8	Ruggedness (% RSD analyst to analyst variation)	98.19-98.02%	98.39-100.725

aSD = Standard deviation, bLOD = Limit of detection, cLOQ = Limit of quantification, dRSD = Relative standard deviation.

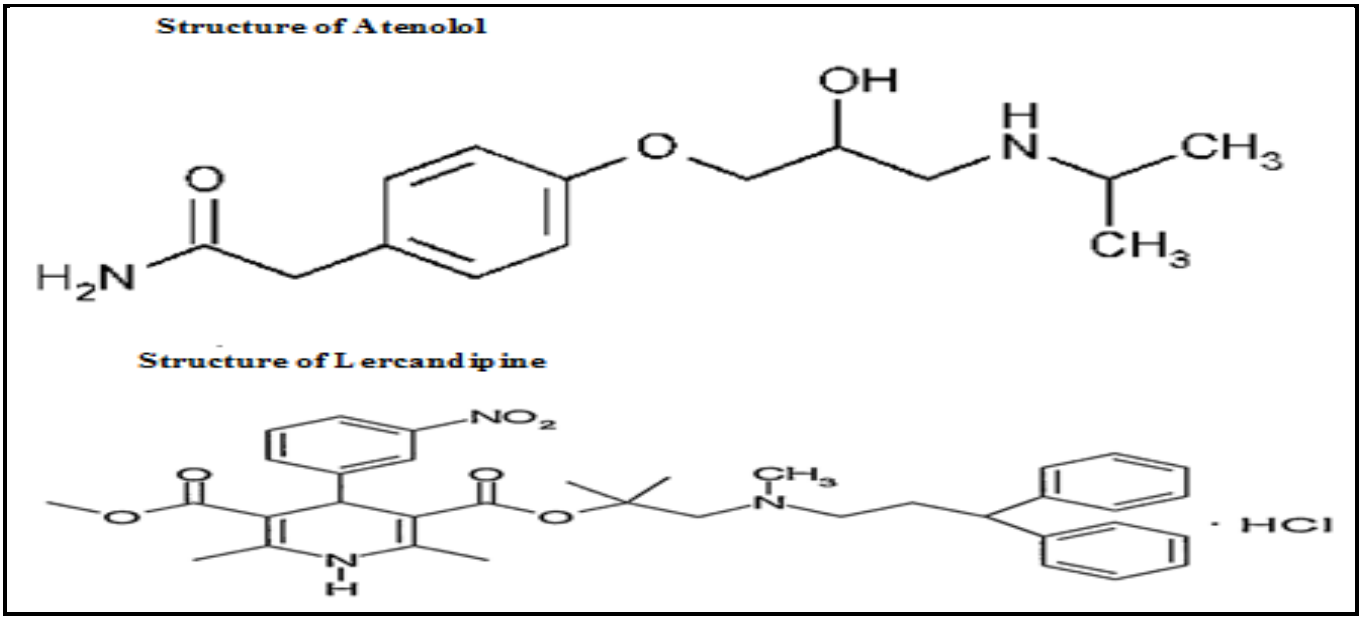
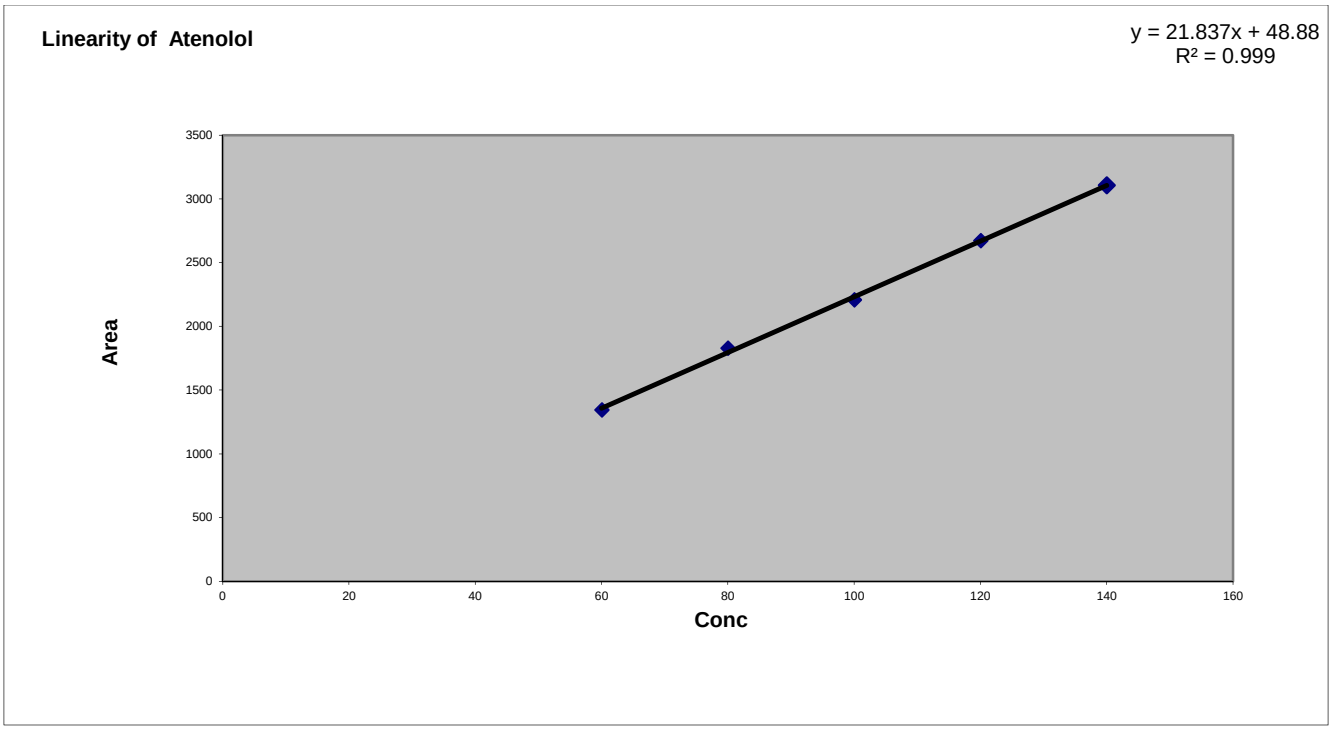


Figure No.1: Structure of Atenolol and Lercandipine



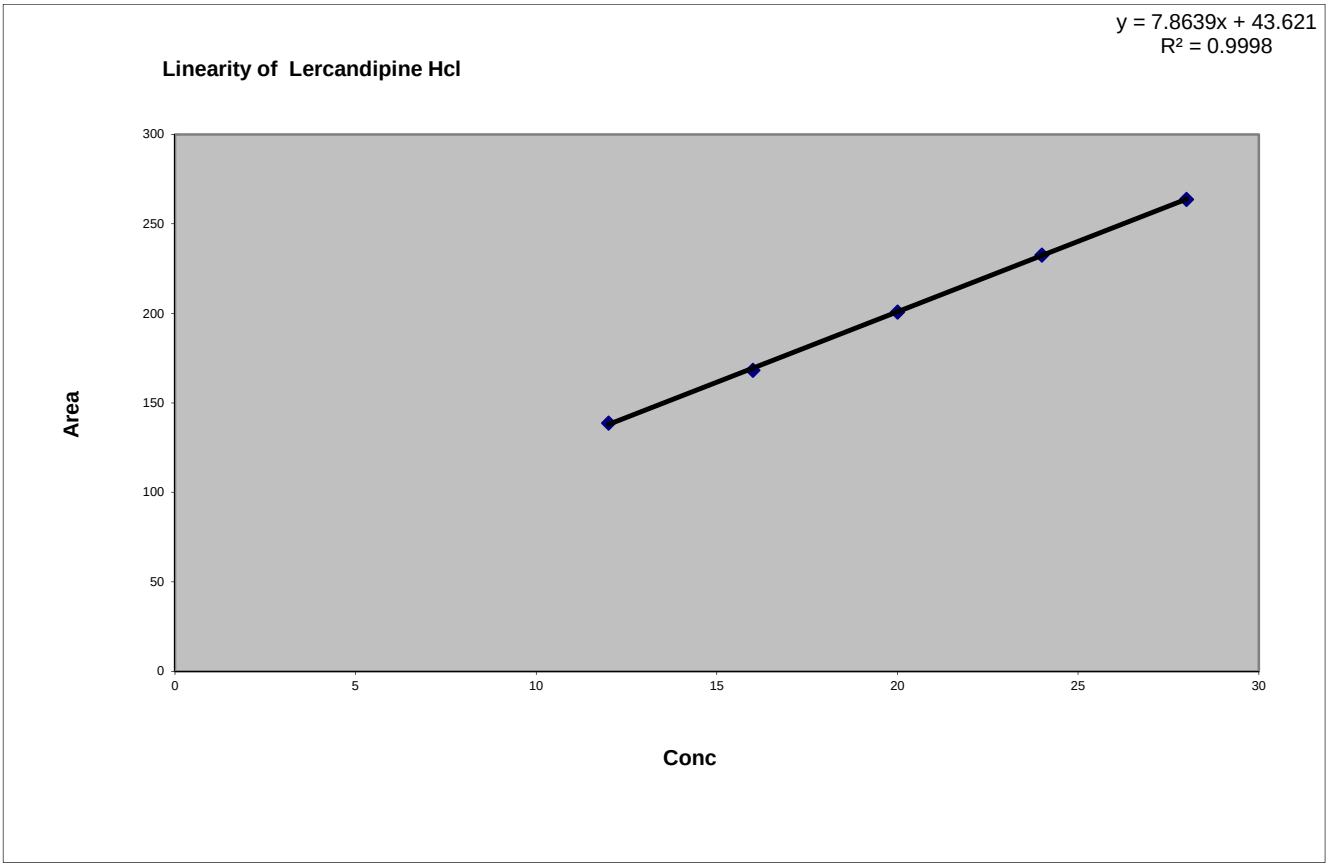


Figure No.2: Linearity (calibration) curve of Atenolol and Lercandipine

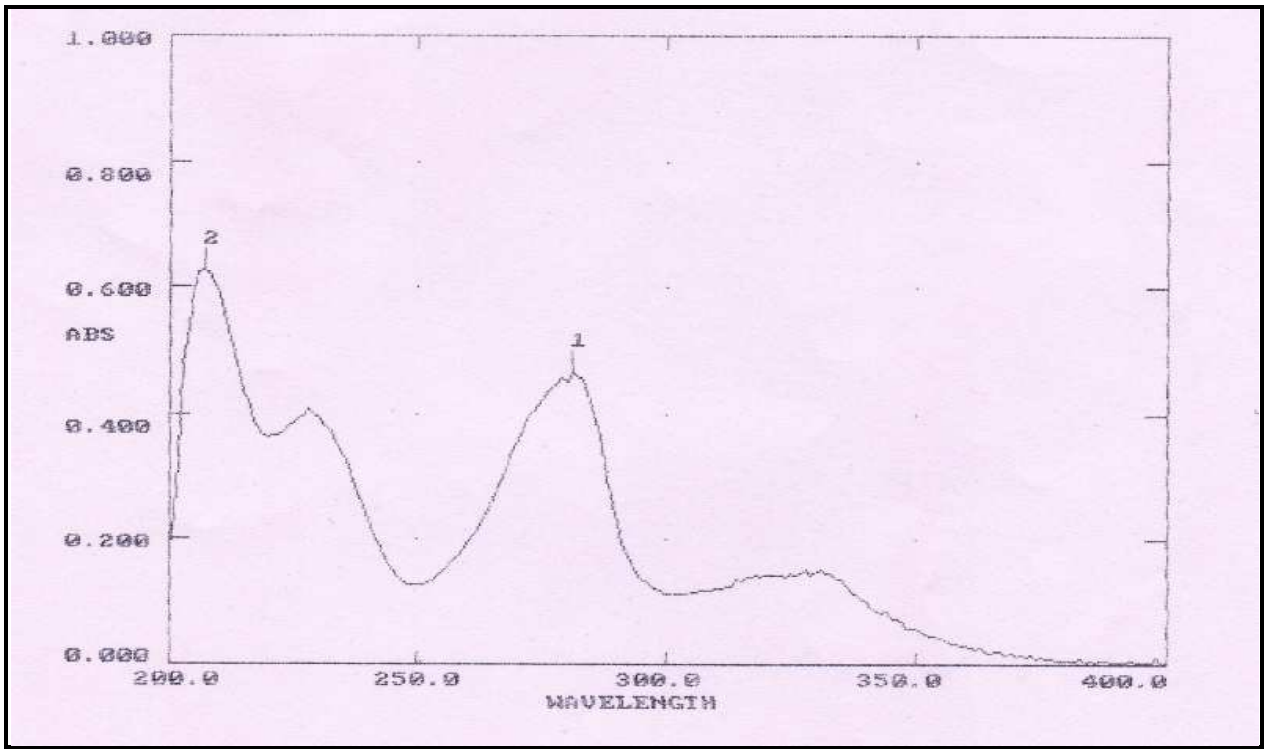


Figure No.3: Determination of working wavelength (λmax) of Atenolol

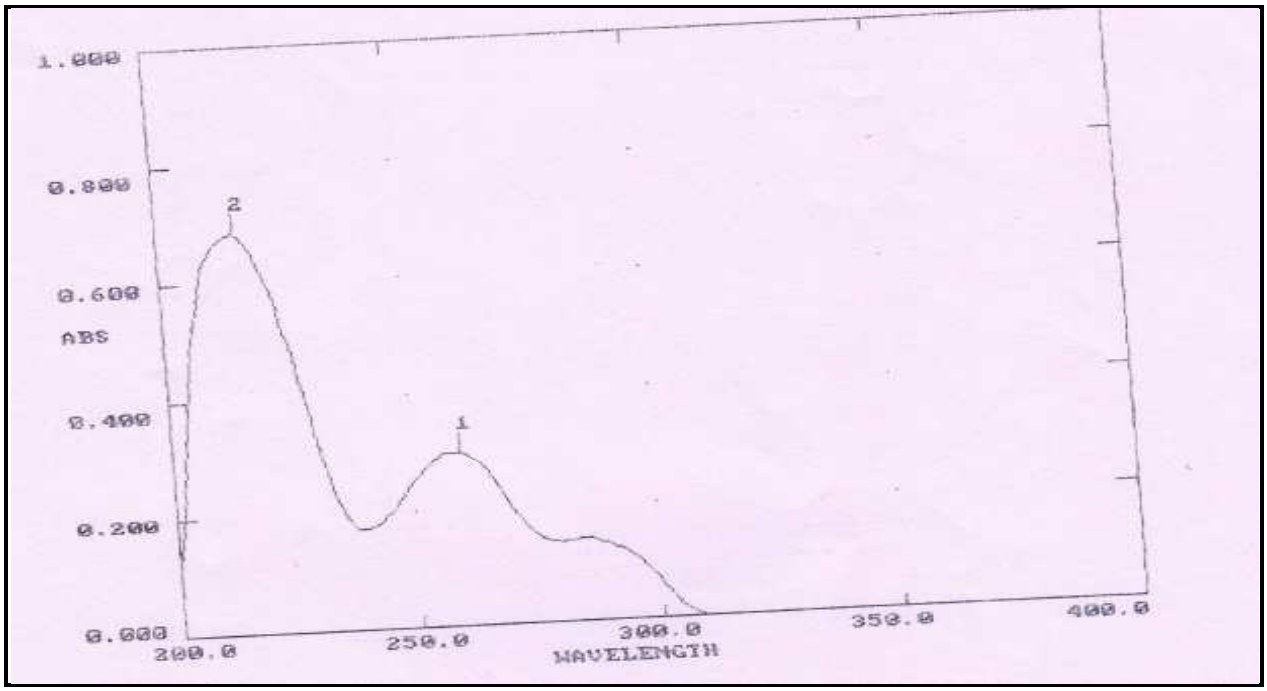


Figure No.4: Determination of working wavelength (λmax) of Lercandipine

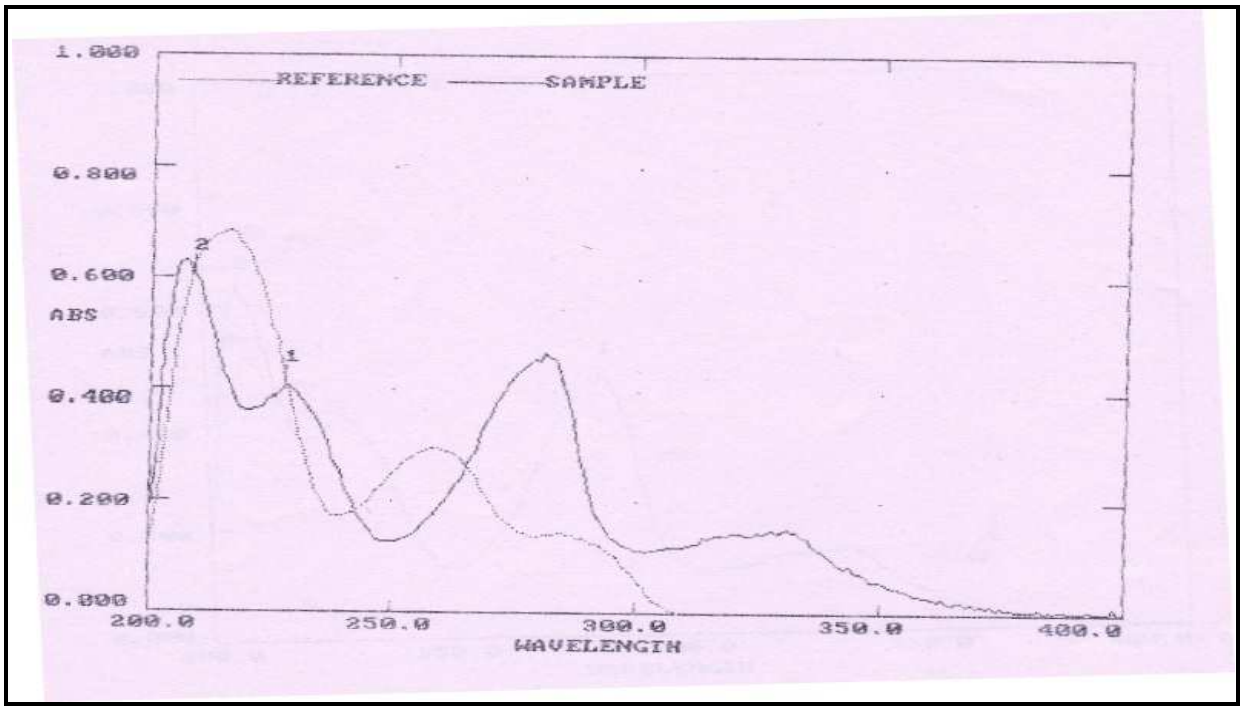


Figure No.5: Determination of Isobestic Point

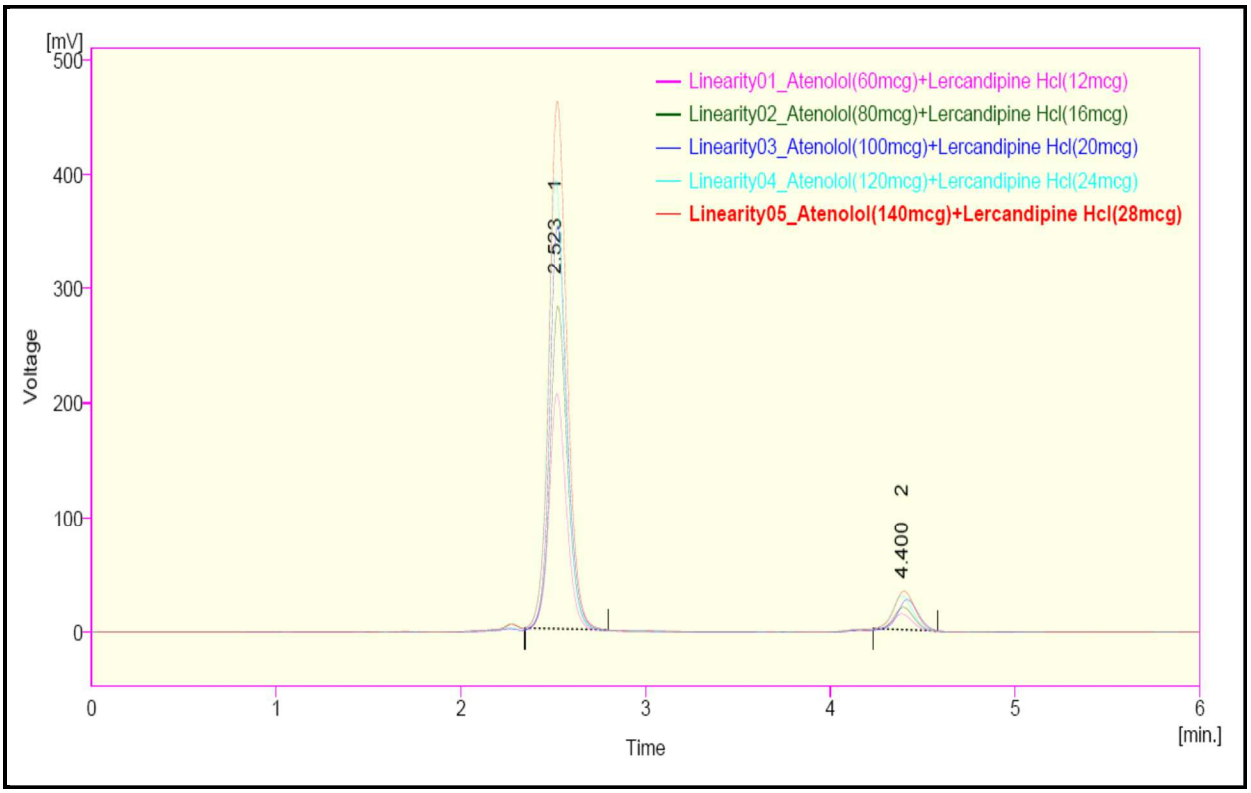


Figure No.6: Overlain chromatograms of Linearity

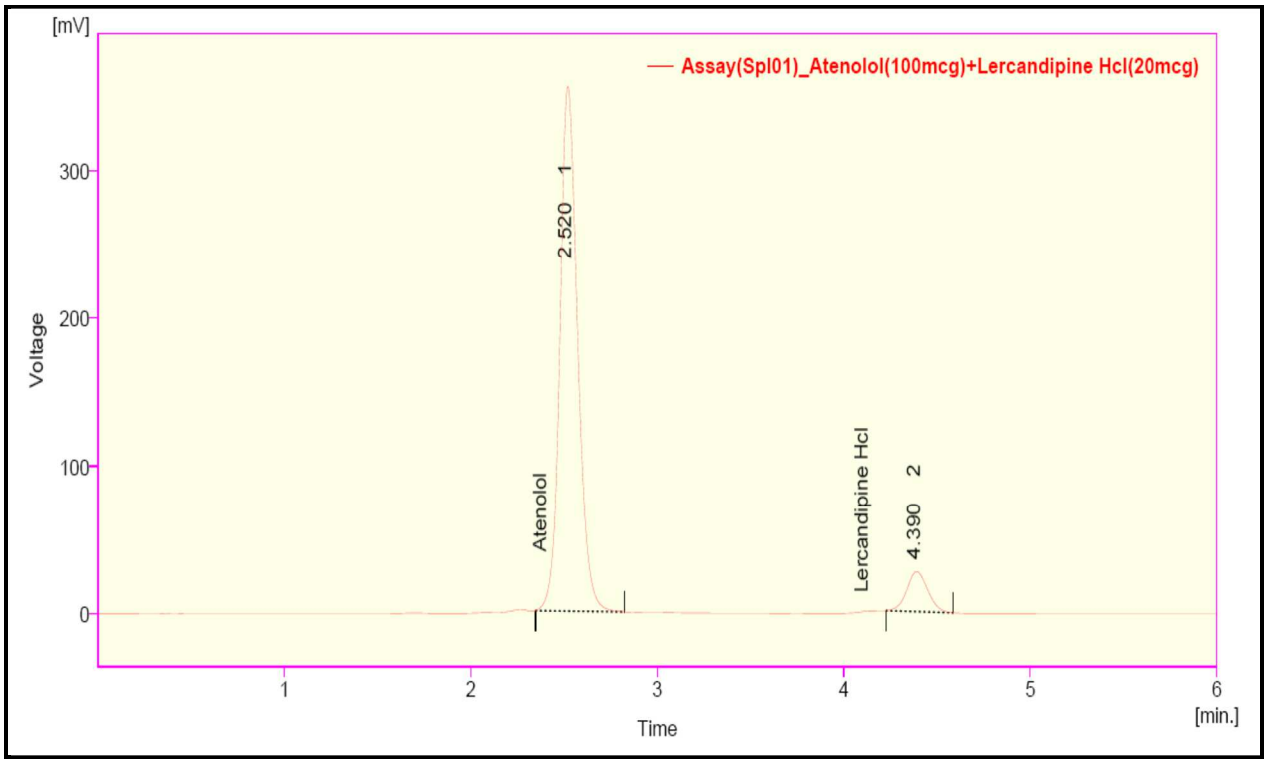


Figure No.7: Chromatogram of Assay sample preparation

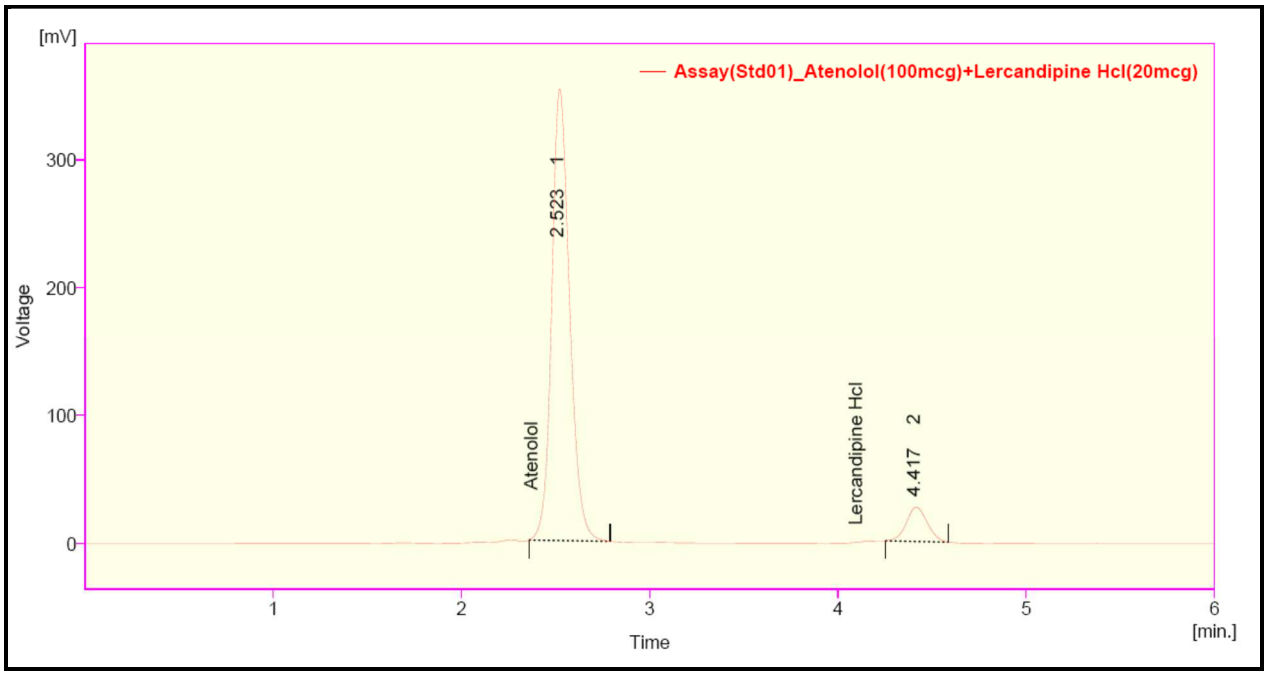


Figure No.8: Chromatogram of Assay standard preparation

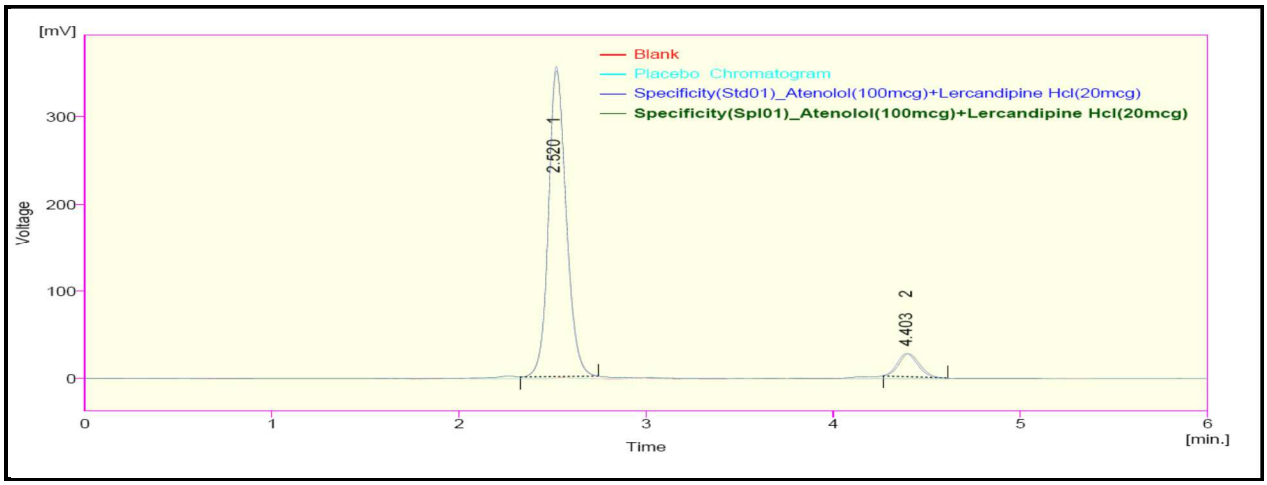


Figure No.9: Overlain chromatograms of Specificity (placebo, blank, sample and standard preparations)

CONCLUSION

The proposed Simultaneous Estimation by RP-HPLC method was found to be simple, sensitive, accurate and precise for determination of Atenolol and Lercandipine in tablet. The method utilizes easily available and cheap solvent for analysis of Atenolol and Lercandipine hence the method was also economic for estimation of Atenolol and Lercandipine from Tablet. The common excipients and other additives are usually present in the Tablet mixtures do not interfere in the analysis of Atenolol and Lercandipine, hence it can be conveniently adopted for routine quality control analysis of the drug in pharmaceutical formulation.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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