A NEW VALIDATED RP-HPLC METHOD FOR THE DETERMINATION OF LERCANIDIPINE AND ATENOLOL IN BULK AND PHARMACEUTICAL DOSAGE FORM

SYED HAFEEZ AHMED, UMME AYMAN
Department of Pharmaceutical Analysis and Quality Assurance
Shadan college of Pharmacy, Hyderabad,Telangana.

ABSTRACT: ***The objective of the present research work was to develop an innovative, simple, and economic method for estimation of Lercanidipine and Atenolol in bulk and dosage form by RP-HPLC.The chromatographic conditions were performed on Waters ODS (C18) RP Column, 250 mm x 4.6 mm. 5µm as stationary phase and mobile phase was prepared Acetonitrile: Buffer pH-3.4 with OPA (40:60) and other conditions optimized were: flow rate (1.0 ml/minute), wavelength (330 nm), Run time was maintained at 10 minutes.The analytical method is valid for estimation of Lercanidipine and Atenolol over a range of 10 µg/ml–50 µg/ml. The results of system suitability test, linearity, precision and accuracy, robustness, specificity, LOD and LOQ and stabilities presented in this report are within the acceptance range.A specific, sensitive, economic method estimation of Lercanidipine and Atenolol has been developed based on ICH Guidelines with bulk and dosage forms.***

Key Words: Lercanidipine and Atenolol , HPLC, Method Development , ICH, Validation , Accuracy, Precision.

I. INTRODUCTION

Lercanidipine is a calcium channel blocker of the dihydropyridine class. It is sold under various commercial names including Zanidip.It is used alone or with an angiotensin-converting enzyme inhibitor, to treat hypertension.

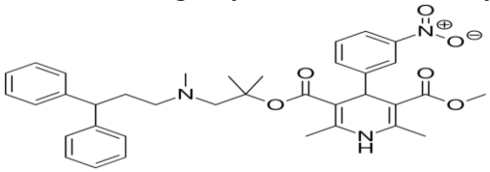

Fig.1. Structure of Lercanidipine

Atenolol is a cardioselective beta-blocker used in a variety of cardiovascular conditions. Sir James Black, a scottish pharmacologist, pioneered the use of beta-blockers for the management of angina pectoris in 1958 for which he received the Nobel Prize.

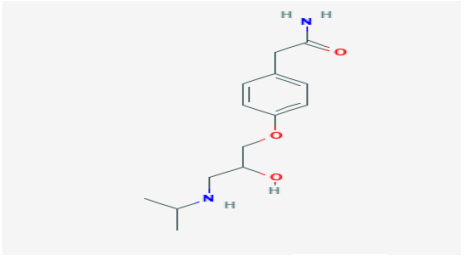

Fig.2. Structure of Atenolol

II. EXPERIMENTAL

2.1 Materials and Methods:

Pharmaceutical grade working standard Lercanidipine and Atenolol were obtained from Syncorp Pvt. Laboratories, Hyderabad, India. All chemicals and reagents were HPLC grade and were purchased from S D Fine-Chem Limited & Loba Chemie Pvt Ltd, Mumbai, India.

2.2 Instrumentation:

The analysis was performed using HPLC (Waters-717 series) with PDA detector and data handling system EMPOWER2 software, UV-Visible double beam spectrophotometer (ELICO SL-159), analytical balance 0.1mg Sensitivity (SHIMADZU), pH meter (Labindia), ultra sonicator. The column used is Symmetry ODS RP C_{18},5µm, 15mm x 4.6mm i.d. (as Stationary phase) with the flow rate 1.0ml/min (isocratic).

2.3 Sample & Standard Preparation for the Analysis

25 mg of Lercanidipine and Atenolol standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.1ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

2.4 Selection of wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent. (After optimization of all conditions) for UV analysis. It is scanned in the UV spectrum in the range of 200 to 400nm. While scanning the Lercanidipine and Atenolol solution we observed the maxima at 330 nm.

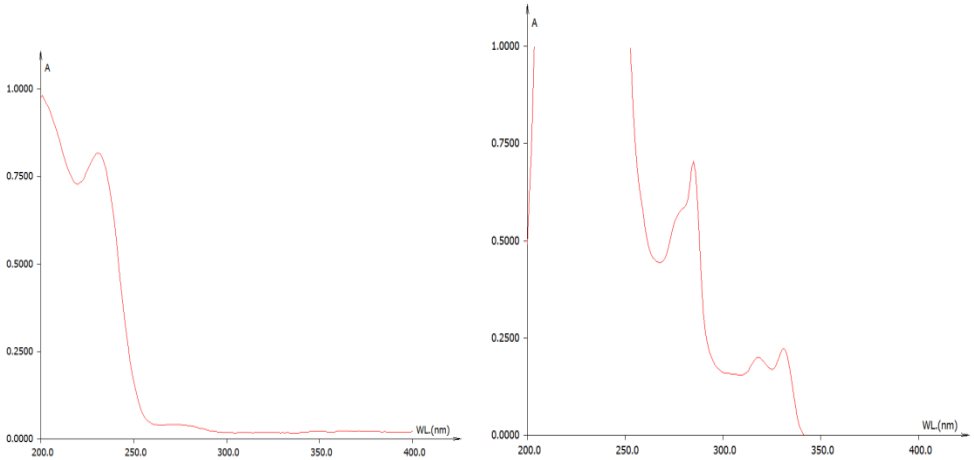

Fig.3. UV Spectrum for Lercanidipine & Atenolol

2.5 Method Development

2.5.3 Summary of Optimized Chromatographic Conditions:

The Optimum Chromatographic conditions obtained from experiments can be summarized as below:

Table-1: Summary of Optimised Chromatographic Conditions

Mobile phase	**Acetonitrile : Buffer pH-3.4 with OPA (40:60)**
Wavelength	330 nm
Flow rate	1.0 ml/ min.
Auto Sampler Temperature	Ambient
Injection Volume	20µl
Run time	10 min.
Column	Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.
Column Temperature	Ambient

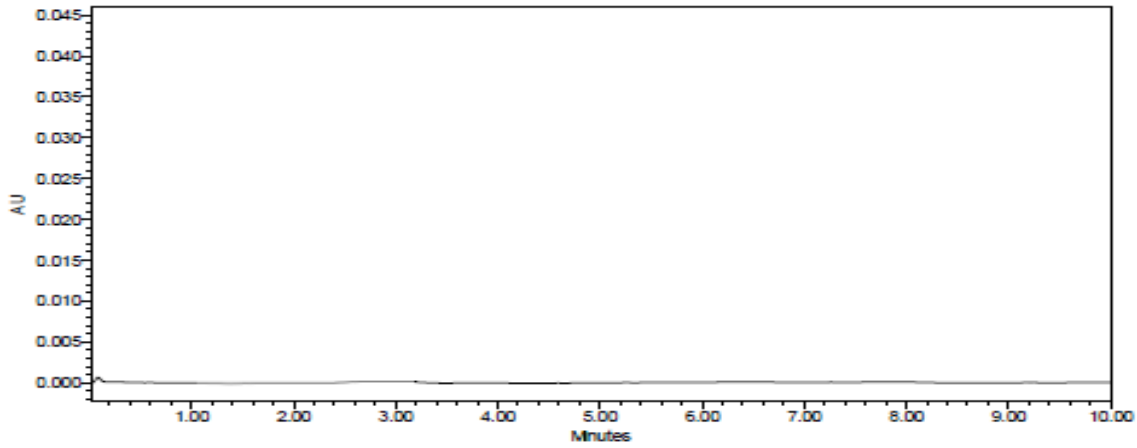

Fig.4. Chromatogram for Blank Preparation

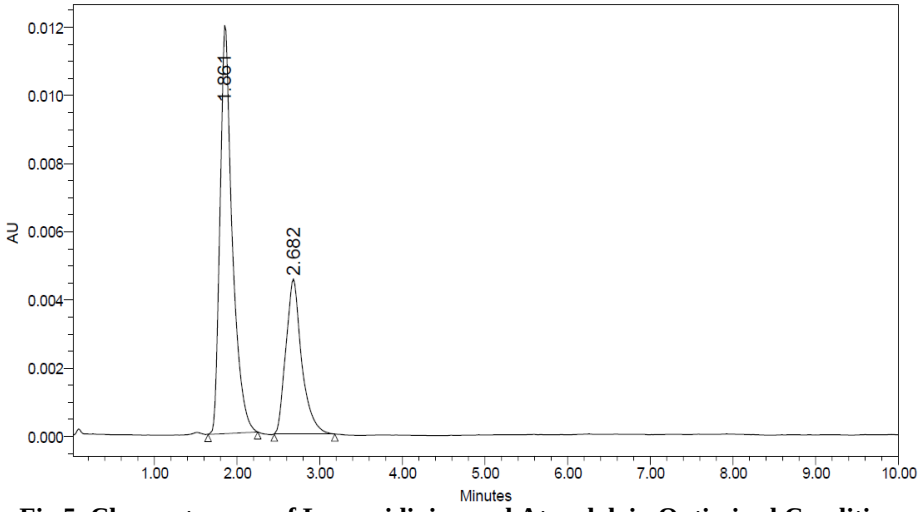

Fig.5. Chromatogram of Lercanidipine and Atenolol in Optimized Condition

2.6 Method validation:

2.6.1 Linearity & Range: Lercanidipine

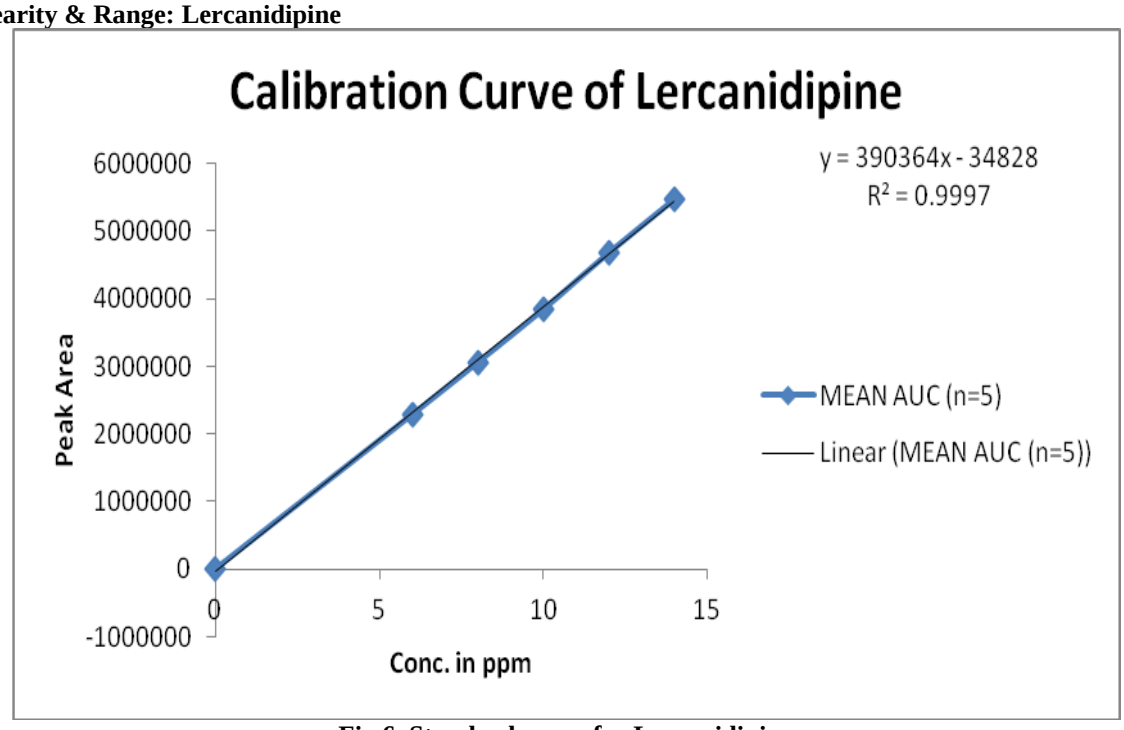

Fig.6. Standard curve for Lercanidipine

Table-2: Linearity Results for Lercanidipine

CONC. (µg/ml)	AUC (n=6)
0	0
6	2281962
8	3053421
10	3837632
12	4673649
14	5462556

Atenolol

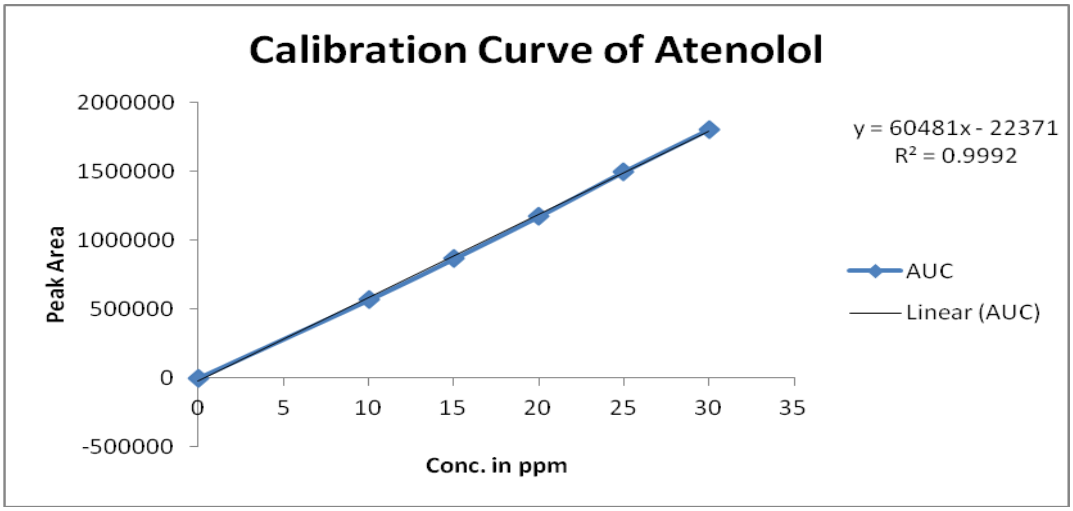

Fig.7. Standard curve for Atenolol

Table-3: Linearity Results for Atenolol

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
10	567458
15	865310
20	1174123
25	1500209
30	1806775

2.6.2. Accuracy:

Table-4: Accuracy Results for Lercanidipine

Sample ID	Concentration (µg/ml)			% Recovery of Pure drug	Statistical Analysis
	Conc. Found	Conc. Recovered	Peak Area		
S_1 : 80 %	8	8.039	348673	100.487	Mean= 100.633% S.D. = 0.182066 % R.S.D.= 0.180921
S_2 : 80 %	8	8.046	348945	100.575	
S_3 : 80 %	8	8.067	349745	100.837	
S_1 : 100 %	10	9.862	419823	98.62	Mean= 99.95% S.D. = 1.340112% R.S.D.= 1.340782
S_2 : 100 %	10	9.993	424941	99.93	
S_3 : 100 %	10	10.130	430295	101.3	
S_7 : 120 %	12	12.115	507788	100.958	Mean= 100.9717% S.D. = 0.512637 % R.S.D.= 0.507703
S_8 : 120 %	12	12.179	510262	101.491	
S_9 : 120 %	12	12.056	505468	100.466	

Table-5 : Accuracy Results for Atenolol

Sample ID	Concentration (µg/ml)			% Recovery of Pure drug	Statistical Analysis
	Conc. Found	Conc. Recovered	Peak Area		
S_1 : 80 %	16	15.991	989572	99.943	Mean= 100.1577% S.D. = 0.654939 % R.S.D.= 0.653908
S_2 : 80 %	16	16.143	998756	100.893	
S_3 : 80 %	16	15.942	986589	99.637	
S_4 : 100 %	20	19.995	1231734	99.975	Mean= 100.795% S.D. = 0.822511% R.S.D.= 0.816024
S_5 : 100 %	20	20.158	1241569	100.79	
S_6 : 100 %	20	20.325	1251694	101.62	
S_7 : 120 %	24	24.335	1494218	101.395	Mean= 100.805% S.D. = 0.613739 % R.S.D.= 0.608837
S_8 : 120 %	24	24.204	1486312	100.85	
S_9 : 120 %	24	24.041	1476398	100.170	

2.6.3. Precision:

2.6.3.1. Repeatability

Table-6: Repeatability Results of Lercanidipine and Atenolol

Concentration of Lercanidipine and Atenolol in ppm	Rt of Lercanidipine	Peak area of Lercanidipine	Rt of Atenolol	Peak area of Atenolol
10 +10	2.264	3303800	3.132	951802
10 +10	2.246	3349883	3.132	958267
10 +10	2.264	3353514	3.129	954481
10 +10	2.246	3384162	3.113	952151
10 +10	2.280	3390496	3.113	952308
AVG	**2.26**	**3356371**	**3.1238**	**953801.8**
S.D.	**0.014353**	**34463.10324**	**0.009935**	**2709.017**
% RSD	**0.635075**	**1.026796598**	**312.38**	**0.284023**

2.6.3.2. Intermediate precision:

Table-7: Results of intra-assay & inter-assay

Conc. Of Lercanidipine (API) (µg/ml)	Observed Conc. Of Lercanidipine (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
8	8.09	0.97	8.03	0.96
10	10.05	0.45	10.04	0.47
12	11.98	0.37	11.90	0.12

Table- 8: Data for Atenolol intra-assay & inter-assay analysis

Conc. Of Atenolol (API) (µg/ml)	Observed Conc. Of Atenolol (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
8	7.97	0.27	8.09	0.59
10	10.14	1.29	9.95	0.64
12	12.08	0.61	11.94	0.26

2.6.4. Method Robustness:

Table-9: Result of Method Robustness Test

Change in parameter	**% RSD**
Flow (1.1 ml/min)	1.05
Flow (0.9 ml/min)	0.67
Temperature (27^0C)	0.58
Temperature (23^0C)	0.61
Wavelength of Detection (332 nm)	0.38
Wavelength of detection (328 nm)	0.17

Table-10 : Result of method robustness test

Change in parameter	**% RSD**
Flow (1.1 ml/min)	0.09
Flow (0.9 ml/min)	0.07
Temperature (27^0C)	0.28

Temperature (23^0C)	0.74
Wavelength of Detection (332 nm)	0.86
Wavelength of detection (328 nm)	0.67

2.6.5. LOD & LOQ:lOD: The values were evaluated based on relative standard deviation of response and slope of the calibration curve of Lercanidipine and Atenolol .

Observation: The Minimum concentration level at which the analyte can be reliable detected LOD & quantified LOQ were found to be 0.09 & 0.29 µg/ml respectively for Lercanidipine .

The LOD was found to be 0.1 µg/ml and LOQ was found to be 0.3 µg/ml for Atenolol which represents that sensitivity of the method is high.

2.6.6-ASSAY OF LERCANIDIPINE AND ATENOLOL TABLETS

Table-13: Assay of Lercanidipine & Atenolol Tablets

Brand name of tablets	Labelled amount of Drug (mg) Lercanidipine & Atenolol	Mean (±SD) amount (mg) found by the proposed method (n=6)	Mean (± SD) Assay (n = 6)
LOTENSYL-AT tab	10/50	9.78 (±0.08)/49.22 (±0.05)	99.78(±0.48) /99.77(±0.12)

2.6.8 Stability Studies:

The various degradation pathways studied are acid hydrolysis, basic hydrolysis, thermal degradation, photolytic degradation and oxidative degradation.

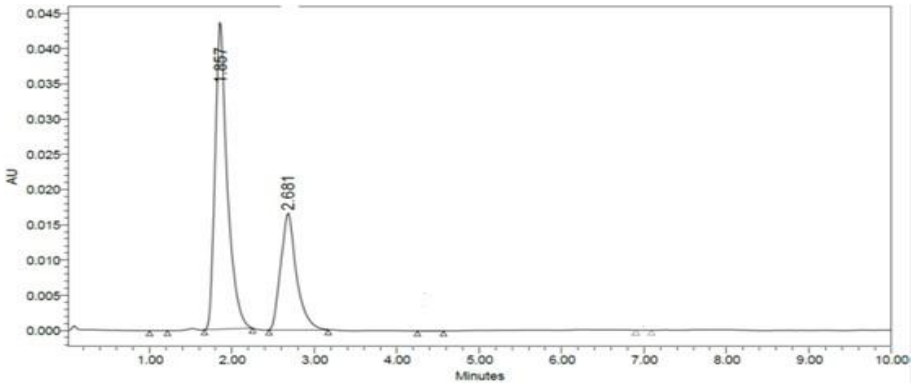

Fig.8. Chromatogram for Acid Degradation

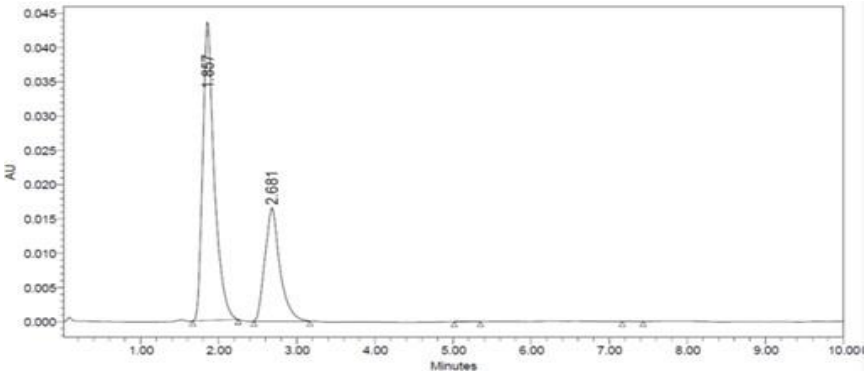

Fig.9. Chromatogram for Basic Degradation

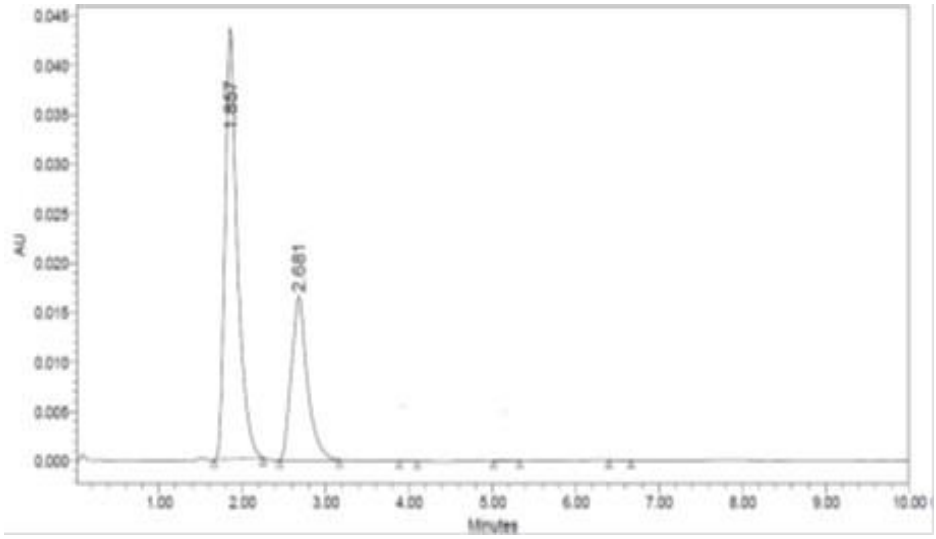

Fig.10. Chromatogram for Thermal Degradation

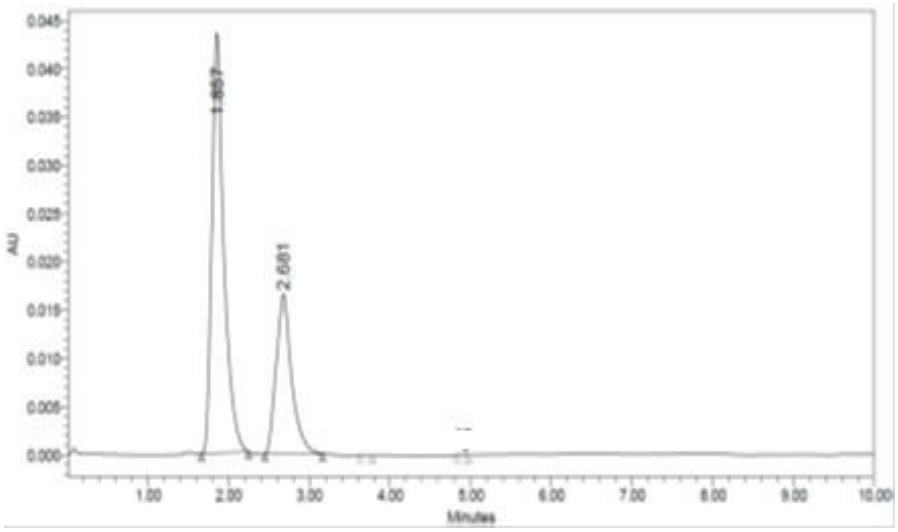

Fig.11. Chromatogram for Photolytic Degradation

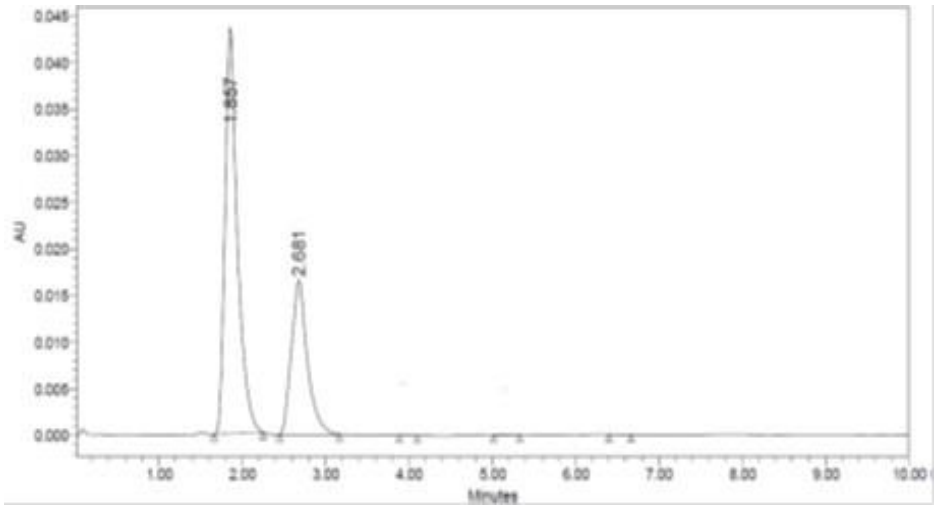

Fig.12. Chromatogram for Oxidation with 3% H_2O_2 Degradation

Table 14-: Results of forced degradation studies of Lercanidipine and Atenolol API.

Stress condition	Time (hours)	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1N HCl)	24Hrs.	76.52	23.48	100.00
Basic Hydrolysis (0.IN NaOH)	24Hrs.	79.37	20.63	100.00
Thermal Degradation (50 ^{0}C)	24Hrs.	90.41	9.59	100.00
UV (254nm)	24Hrs.	99.21	0.79	100.00
3% Hydrogen peroxide	24Hrs.	81.62	18.38	100.00

III. RESULTS

The optimized chromatographic conditions were Waters ODS (C18) RP Column, 250 mm x 4.6 mm. 5µm as stationary phase and mobile phase was prepared with) Acetonitrile: Buffer pH-3.4 with OPA (40:60) and other conditions optimized were: flow rate (1.0 ml/minute), wavelength (330 nm), Run time was maintained at 10 minutes.

In these chromatographic conditions the peak was pure, sharp, symmetric and found a greater number of theoretical plates.

The results obtained in method validation were :

Linearity & Range: Linearity range was found to be 0-14 µg/ml for Lercanidipine and 0-30 µg/ml for Atenolol. The correlation coefficients were found to be 0.999 & 0.999, the slopes were found to be 39036 & 60481 and intercept were found to be 34828 & 22371 for Lercanidipine and Atenolol respectively.

Accuracy: The mean recoveries were found to be 100.016, 100.403 and 100.605 % for capecitabine and 100.120, 101.44 and 101.27 % for Temozolamide . The limit for mean % recovery is 98-102% and as both the values are within the limit, hence it can be said that the proposed method was accurate.

Repeatability: The repeatability study which was conducted on the solution having the concentration of about 10 g/ml for Capecitabine and 10 g/ml for Temozolamide (n 6) showed a RSD of 1.026796598% for Capecitabine and 0.284023 % for Temozolamide. It was concluded that the analytical technique showed good repeatability.

LOD & LOQ: The LOD was found to be 0.09 g/ml and 0.12 g/ml for Capecitabine and Temozolamide respectively.

The LOQ was found to be 0.15 g/ml and 0.30 g/ml for Capecitabine and Temozolamide respectively.

Assay: The assay of Dulane-M Tablets containing Lercanidipine was found to be 9.78 (±0.08) and Atenolol was found to be 49.22 (±0.05) and the % purity of the Lercanidipine & Atenolol was found to be 99.78(±0.48) /99.77(±0.12).

Degradation studies: The results of the stress studies indicated the specificity of the method that has been developed. Lercanidipine & Atenolol was more stable in thermal and peroxide stress conditions as compare to other stress conditions.

IV.DISCUSSION

To develop a precise, linear, specific RP-HPLC method for analysis of Lercanidipine & Atenolol, different chromatographic conditions were applied & the results observed were compared with the methods available in literatures.

Deepak Kumar Jain, et al, simultaneous estimation of Atenolol (ATL) and Lercanidipine Hydrochloride (LER) present in tablet dosage forms. Chromatographic separation achieved isocratically on Luna C18 column (5 µm, 150mm x 4.60mm) and ACN/phosphate buffer (60:40, v/v, pH 3.6) as mobile phase, at a flow rate of 0.5ml/min. Detection was carried out at 235 nm. Linearity for ATL and LER were in the range of 50-250 mg/ml and 10- 50 mg/ml respectively. Parameters such as linearity, precision, accuracy, recovery, specificity and ruggedness are studied as reported in the ICH guidelines. The retention times for ATL and LER was found to be 2.27 and 5.97 min respectively.

V. CONCLUSION

A sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of Lercanidipine & Atenolol API. Based on peak purity results, obtained from the analysis of samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Lercanidipine & Atenolol indicated that the developed method is specific for the estimation of Lercanidipine & Atenolol. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

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