A VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CILNIDIPINE AND VALSARTAN IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT : The objective of the present research work was to develop an innovative, simple, and economic method for estimation of Cilnidipine and Valsartan 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 Methanol : Phosphate Buffer (0.2 M, pH=3) = 75:25, flow rate (1.0 ml/minute), wavelength (259 nm), Run time was maintained at 08minutes.The analytical method is valid for estimation of Cilnidipine and Valsartan 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 Cilnidipine and Valsartan has been developed based on ICH Guidelines with bulk and dosage forms.

Key Words: Cilnidipine and Valsartan , HPLC, Method Development , ICH, Validation , Accuracy, Precision.

I. INTRODUCTION

Cilnidipine is a dihydropyridine calcium antagonist. Compared with other calcium antagonists, cilnidipine can act on the N-type calcium channel that existing sympathetic nerve end besides acting on L-type calcium channel that similar to most of the calcium antagonists.

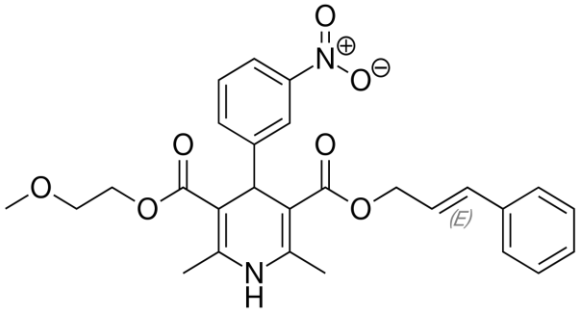

Fig.1. Structure of Cilnidipine

Valsartan belongs to the angiotensin II receptor blocker (ARB) family of drugs, which also includes telmisartan, candesartan, losartan, olmesartan, and irbesartan.

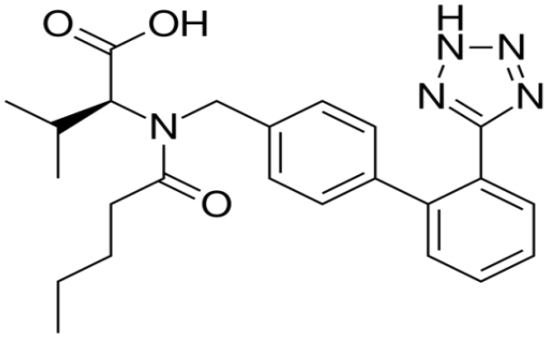

Fig.2. Structure of Valsartan

II. EXPERIMENTAL

2.1 Materials and Methods:

Pharmaceutical grade working standard Cilnidipine and Valsartan 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 Cilnidipine and Valsartan 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 Cilnidipine and Valsartan solution we observed the maxima at 259 nm.

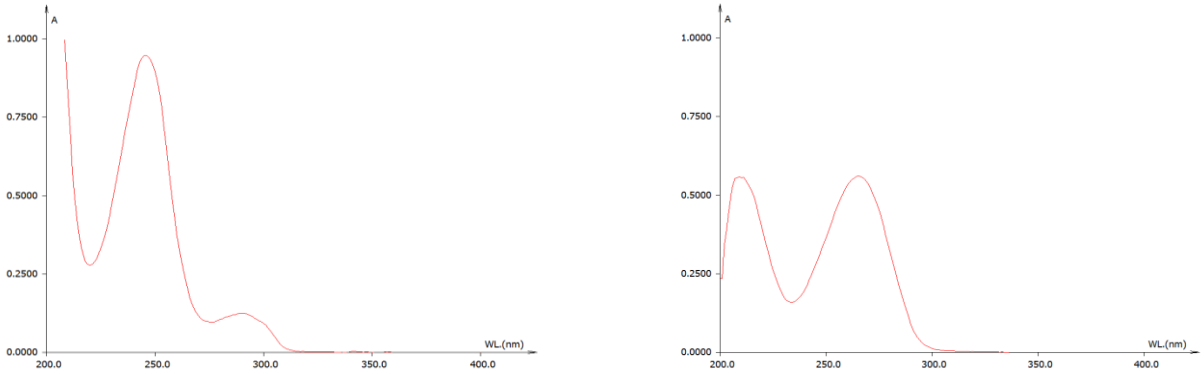

Fig.3. UV Spectrum for Cilnidipine and Valsartan

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	Methanol : Phosphate Buffer (0.2 M, pH=3) = 75:25
Column	Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.
Column Temperature	Ambient
Detection Wavelength	259 nm
Flow rate	1.0 ml/ min.
Run time	08 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	10µl
Type of Elution	Isocratic

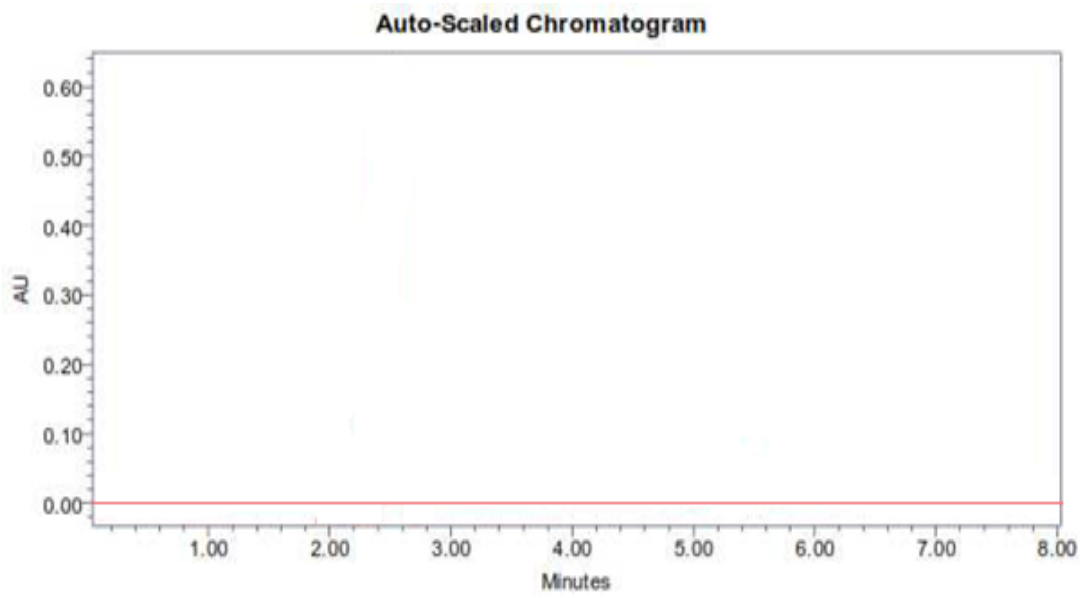

Fig.4. Chromatogram for Blank Preparation

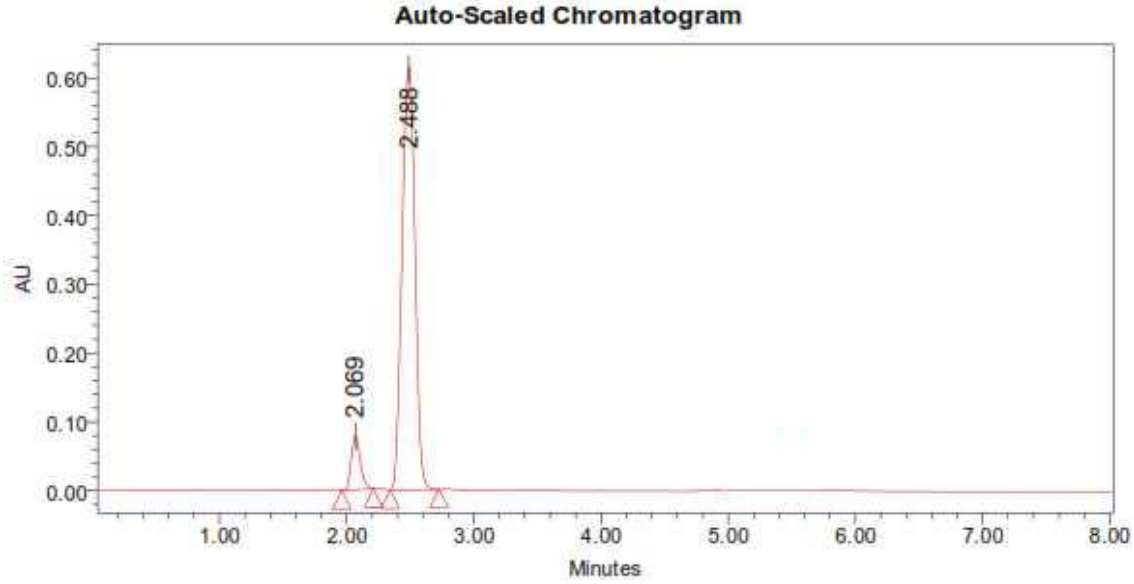

Fig.5. Chromatogram of Cilnidipine and Valsartan in Optimized Condition

2.6 Method validation:

2.6.1 Linearity & Range: Cilnidipine

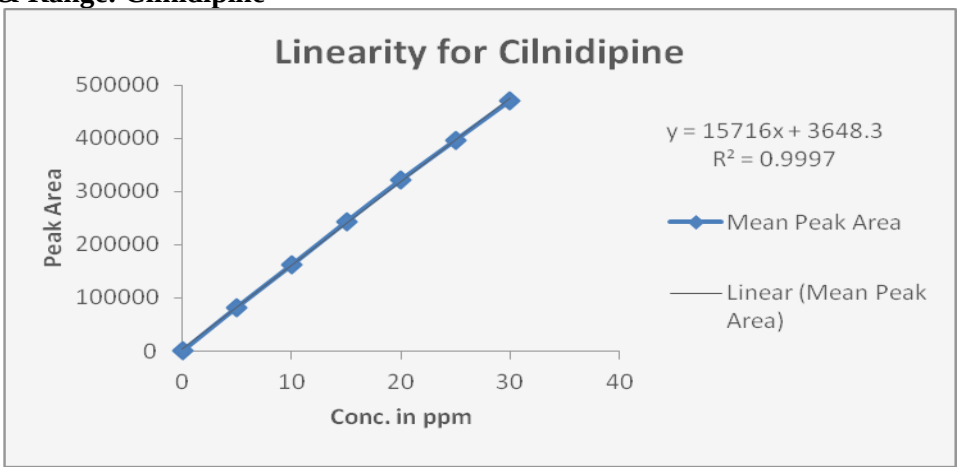

Fig.6. Standard curve for Cilnidipine

Table-2: Linearity Results for Cilnidipine

CONC. (µg/ml)	AUC (n=6)
0	0
5	82442
10	161724
15	242754
20	321606
25	396371
30	470843

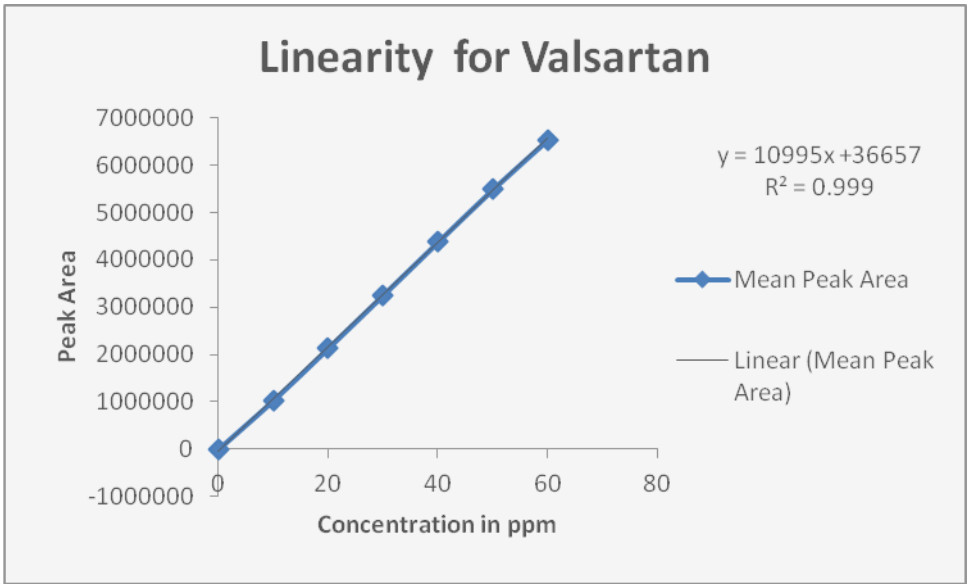

Fig.7. Standard curve for Valsartan

Table-3: Linearity Results for Valsartan

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
10	1031032
20	2135302
30	3255282

40	4379382
50	5493754
60	6539365

2.6.2. Accuracy:

Table-4: Accuracy Results for Cilnidipine

Sample ID	Concentration (µg/ml)			%Recovery of Pure drug	Statistical Analysis
	Conc. Found	Conc. Recovered	Peak Area		
S_1 : 80 %	12	11.974	191834	99.783	Mean= 99.769% S.D. = 0.296248 % R.S.D.= 0.296934
S_2 : 80 %	12	11.936	191235	99.466	
S_3 : 80 %	12	12.007	192358	100.058	
S_4 : 100 %	15	15.243	243212	101.62	Mean= 100.8887% S.D. = 1.044048 R.S.D.= 1.034852
S_5 : 100 %	15	15.203	242581	101.353	
S_6 : 100 %	15	14.954	238673	99.693	
S_7 : 120 %	18	17.899	284962	99.438	Mean= 100.9737% S.D. = 1.331212 % R.S.D. = 1.318376
S_8 : 120 %	18	18.324	291643	101.8	
S_9 : 120 %	18	18.303	291312	101.683	

Table-5: Accuracy Results for Valsartan

Sample ID	Concentration (µg/ml)			%Recovery of Pure drug	Statistical Analysis
	Conc. Found	Conc. Recovered	Peak Area		
S_1 : 80 %	24	24.186	191834	100.775	Mean= 100.779% S.D. = 0.406015 % R.S.D.= 0.402876
S_2 : 80 %	24	24.090	191235	100.375	
S_3 : 80 %	24	24.285	192358	101.187	
S_4 : 100 %	30	29.932	365768	99.773	Mean= 99.45533% S.D. = 0.293933 % R.S.D.= 0.295542
S_5 : 100 %	30	29.820	364532	99.40	
S_6 : 100 %	30	29.758	363851	99.193	
S_7 : 120 %	36	35.696	429135	99.155	Mean= 99.57733% S.D. = 0.366784 % R.S.D. = 0.368341
S_8 : 120 %	36	35.914	431534	99.761	
S_9 : 120 %	36	35.934	431756	99.816	

2.6.3. Precision:

2.6.3.1. Repeatability

Table-6: Data showing repeatability analysisfor Cilnidipine & Valsartan

HPLC Injection Replicates	AUC for Cilnidipine	AUC for Valsartan
Replicate – 1	249684	3233700
Replicate – 2	249696	3241323
Replicate – 3	246325	3245927
Replicate – 4	249816	3245927
Replicate – 5	249892	3222194
Replicate – 6	249793	3212863
Average	249201	3233655.667
Standard Deviation	1411.088941	13591.6592
% RSD	0.56624	0.420318

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 for Cilnidipine

Change in parameter	% RSD
Flow (0.8 ml/min)	0.45
Flow (1.2 ml/min)	0.38
More Organic	0.87
Less Organic	0.76
Wavelength of Detection (242 nm)	0.99
Wavelength of detection (238 nm)	0.95

Table-10 : Result of Method Robustness Test for Valsartan

Change in parameter	% RSD
Flow (0.8 ml/min)	0.57
Flow (1.2 ml/min)	0.44
More Organic	0.86
Less Organic	0.75
Wavelength of Detection (271 nm)	1.03
Wavelength of detection (267 nm)	0.94

2.6.5. LOD & LOQ:lOD: The LOD was found to be 0.05µg/ml and LOQ was found to be 0.15µg/ml for Cilnidipine respectively which represents that sensitivity of the method is high.

The LOD was found to be 0.09µg/ml and LOQ was found to be 0.27µg/ml for Valsartan respectively which represents that sensitivity of the method is high.

2.6.6 Assay of Cilnidipine And Valsartan Tablets

Table-13: Recovery Data for estimation Cilnidipine and Valsartan

Brand name of Cilnidipine and Valsartan	Labelled amount of Drug (mg)	Mean (± SD) amount (mg) found by the proposed method (n=6)	Assay % (± SD)
Cilnidipine and Valsartan	10/80	9.2 (±0.767) / 98.9 (± 0.559)	99.8(±0.336) / 99.6(± 0.484)

2.6.7 Stability Studies: The various degradation pathways studied are acid hydrolysis, basic hydrolysis, thermal degradation, photolytic degradation and oxidative degradation.

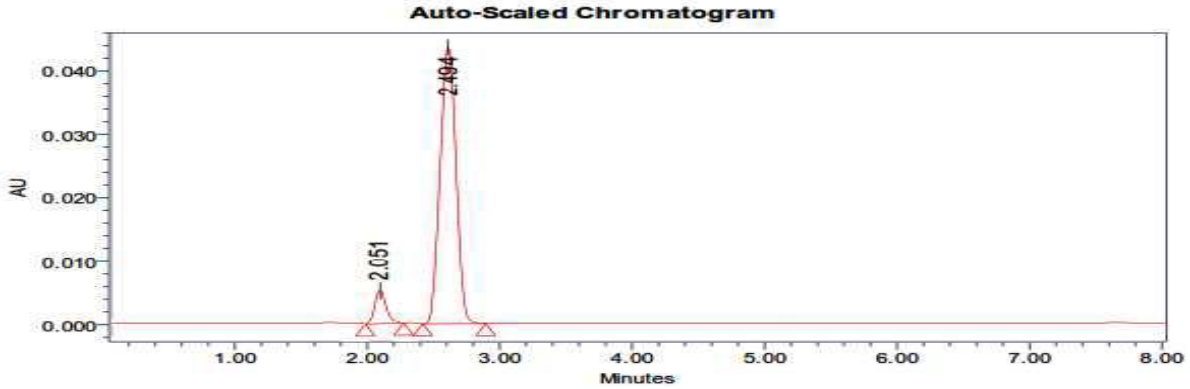

Fig.7. Chromatogram for Acid Degradation

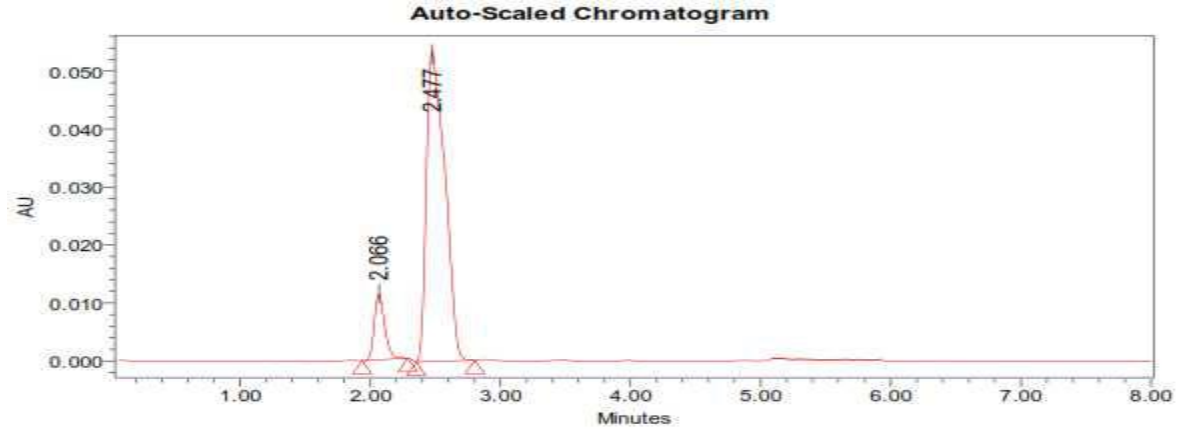

Fig.8. Chromatogram for Basic Degradation

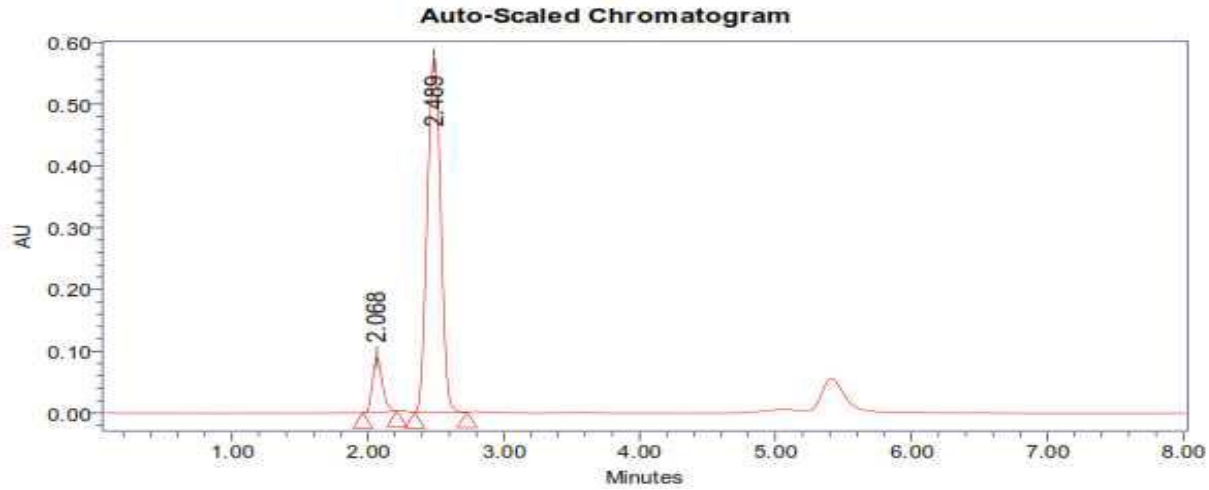

Fig.9. Chromatogram for Thermal Degradation

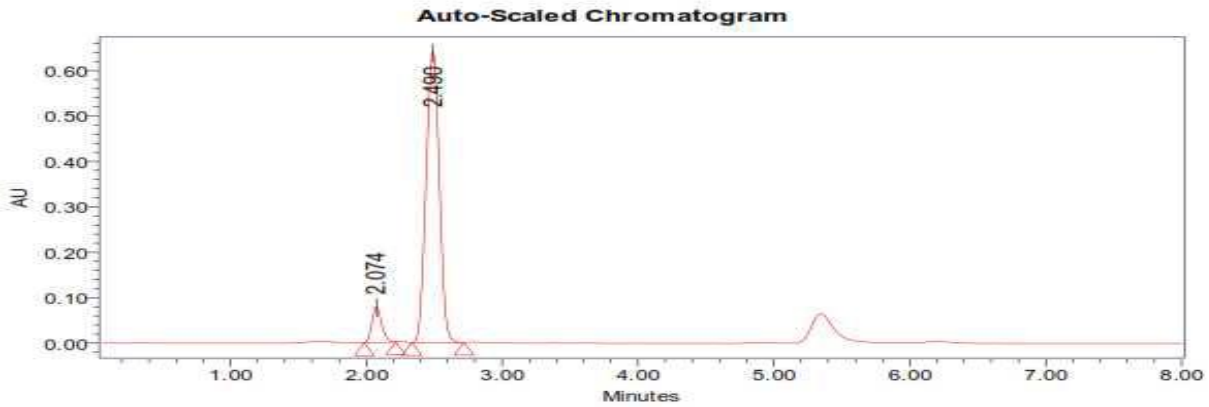

Fig.11. Chromatogram for Photolytic Degradation

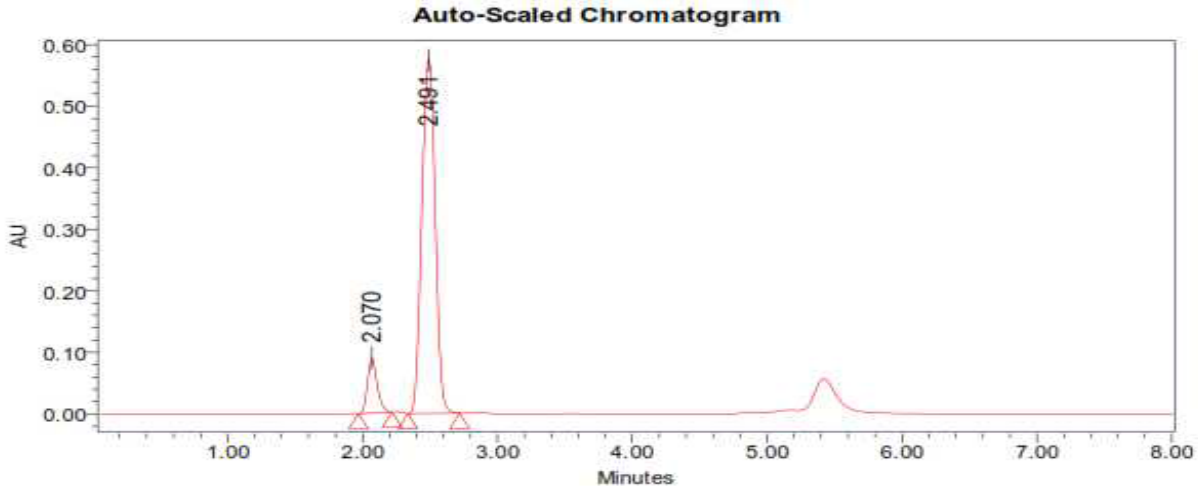

Fig.12. Chromatogram for Oxidation with 3% H_2O_2 Degradation

Table 14-: Results of Force Degradation Studies of Cilnidipine and Valsartan API.

Stress condition	Time (hours)	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1N HCl)	24Hrs.	99.2	0.2	100.00
Basic Hydrolysis (0.IN NaOH)	24Hrs.	98.5	1.5	100.00
Thermal Degradation (60 ^{0}C)	24Hrs.	99.5	0.5	100.00
UV (254nm)	24Hrs.	99.3	0.7	100.00
3% Hydrogen peroxide	24Hrs.	98.4	1.6	100.00

III. RESULTS

The results obtained in method validation were :

Linearity & Range: Linearity range was found to be 5-30 µg/ml for Cilnidipine.The correlation coefficient was found to be 0.999, the slope was found to be 15716 and intercept was found to be 3648 for Cilnidipine .

Linearity range was found to be 10-60 µg/ml for Valsartan .The correlation coefficient was found to be 0.999, the slope was found to be 10995 and intercept was found to be 36657 for Valsartan .

Accuracy: From the Accuracy Method, we observed that the mean %Recovery of the drug are 99.769%, 100.8887% and 100.9737% which is within the range of 98-102% and %RSD is within the range <2 i.e. 0.296934%, 1.034852% and 1.318376% respectively

From the Accuracy Method, we observed that the mean %Recovery of the drug are 100.779%, 99.45533% and 99.57733% which is within the range of 98-102% and %RSD is within the range <2 i.e. 0.402876%, 0.295542% and 0.368341% respectively..

Repeatability: The repeatability study which was conducted on the solution having the concentration of about 15µg/ml for Cilnidipine and 30µg/ml for Valsartan (n =6) showed a RSD of 0.56624% for Cilnidipine and 0.420318% for Valsartan. It was concluded that the analytical technique showed good repeatability.

LOD & LOQ: The LOD was found to be 0.05µg/ml and LOQ was found to be 0.15µg/ml for Cilnidipine respectively which represents that sensitivity of the method is high.

The LOD was found to be 0.09µg/ml and LOQ was found to be 0.27µg/ml for Valsartan respectively which represents that sensitivity of the method is high.

Assay: The assay of of Cilnidipine & Valsartan was found to be 99.8% and 99.6%respectively.

Degradation studies: The results of the stress studies indicated the specificity of the method that has been developed. Cilnidipine & Valsartan 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 Cilnidipine & Valsartan, different chromatographic conditions were applied & the results observed were compared with the methods available in literatures.

Gandla Kumara Swamy, et al, The Present Research study was developed as simple, accurate and precise stability indicating RP-HPLC method has been developed and validated for simultaneous determination of Valsartan and Clinidipine in tablet dosage forms. The chromatographic separation was carried out on an Waters column (150×4.6,i.d 5µ) with a mixture of Acetonitrile : phosphate buffer pH 3.5 adjusted with orthophosphoric acid (70:30, v/v) as mobile phase; at a flow rate of 1.0 ml/min. UV detection was performed at 254 nm. The retention times were 2.33 and 3.55 min. for Valsartan and Clinidipine respectively.

V. CONCLUSION

A sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of Cilnidipine & Valsartan 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 Cilnidipine & Valsartan indicated that the developed method is specific for the estimation of Cilnidipine & Valsartan. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

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