WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 8, 1124-1135 Research Article ISSN 2277– 7105

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING CHROMATOGRAPHIC METHOD FOR SIMULTANEOUS ESTIMATION OF CILNIDIPINE AND IRBESARTAN IN PHARMACEUTICAL DOSAGE FORM

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Article Received on
27 Feb. 2018,

Revised on 19 March 2018,
Accepted on 09 April 2018,

DOI: 10.20959/wjpr20188-11910

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ABSTRACT

A simple, sensitive, accurate, rapid and economical RP-HPLC method was developed and validated for the determination of Irbesartan and Cilnidipine in pharmaceutical dosage form. Chromatography was performed on a Hypersil C_{18} BDS column (25cm x 0.46 cm) and Phosphate Buffer (pH 4.5): Methanol in the ratio (85:15) as mobile phase at a flow rate of 1 ml/min. Wavelength of detection used was 248 nm. The retention time of Cilnidipine and Irbesartan was obtained as 5.343 min and 4.080 min respectively. The obtained calibration curve was linear in the concentration range of Cilnidipine is 0.5-1.5 µg/ml and for Irbesartan is 15-45 µg/ml. The LOD and LOQ was found to be 1.167 µg/ml and 3.538 µg/ml for Irbesartan and 0.046 µg/ml and 0.139 µg/ml for Cilnidipine respectively. The method was successfully applied for the analysis of drugs in pharmaceutical formulation. Results of the analysis were validated statistically and by recovery studies.

KEYWORDS: Irbesartan, Cilnidipine, stability indicating RP-HPLC method, validation.

INTRODUCTION

Cilnidipine is a calcium channel blocker. It inhibits cellular influx of calcium, thus causing vasodilatation. Cilnidipine is chemically 3-(2-methoxyethyl)5-(2E)-3-phenylprop-2-en-1-yl2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate and molecular formula $C_{27}H_{28}N_2O_7$.[1] Irbesartan is used mainly for the treatment of hypertension. Irbesartan (INN) pronounced is an angiotensin II receptor antagonist. Irbesartan IAPUC name is 2-butyl-3-({4-[2-(2*H*-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1,3-diazaspiro[4.4]non-1-en-4-one and molecular formula $C_{25}H_{28}N_6O$.[2] Both drugs used in combination to treat Anti hypertension agent.[3]

Literature review reveals that the no stability indicating simultaneous estimation of Irbesartan and Cilnidipine in combined pharmaceutical dosage form by RP-HPLC.[4] So, develop stability indicating reverse phase high performance liquid chromatographic method for simultaneous estimation of Irbesartan and Cilnidipine in Combined Dosage Form. So develop simple, accurate, rapid and sensitive stability indicating Reverse Phase HPLC method for simultaneous estimation of Irbesartan and Cilnidipine.[5-11]

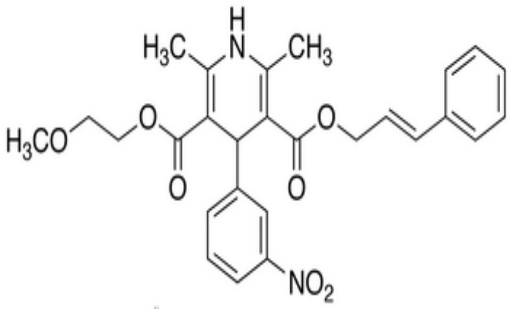

Fig. 1: Chemical structure of cilnidipine.

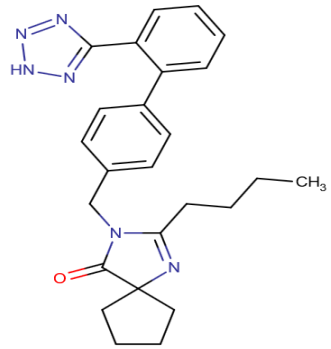

Fig. 2: Chemical structure of irbesartan.

MATERIALS AND METHODS

Materials and reagents

Methanol, water, Potassium Dihydrogen Phosphate of HPLC and AR grade were procured from Bhumi sales. Cilnidipine and Irbesartan standard sample was provided by Yash Pharma, Ahmedabad, India. Clindasartan commercial formulation was procured from local market.

Instrumentation and analytical conditions

The HPLC system consisted of a pump. HPLC system consist of model Shimadzu LC-20 AT having SPD-20A detector and rheodyne injector with 20µl loop volume. Isocratic elution with Phosphate Buffer (pH 4.5): Methanol was used at a flow rate of 1ml/ min. The mobile phase was prepared freshly and degassed by sonicating for 5 min before use. UV spectrophotometer consists of model Systronic 119 is used to measure the wavelength of the solution of Cilnidipine and Irbesartan.

Preparation of standard stock solution

Stock standard solution of 300 µg/ml of Irbesartan was prepared freshly by accurately weighing 30 mg of Irbesartan into 100 ml volumetric flask. Dissolved and made up to the volume with mobile phase.

Stock standard solution of 10 µg/ml of Cilnidipine was prepared freshly by accurately weighing 10 mg of Cilnidipine into 100 ml volumetric flask. Dissolved and made up to the volume with mobile phase. Take 10 ml from this Solution and transfer to 100 ml Volumetric flask and Volume was made up with the Mobile phase.

Preparation of working standard solution

Take 1 ml from the Irbesartan stock solution and 1ml from Cilnidipine stock solution and transferred to 10 ml volumetric flask and volume made up to the mark by mobile phase.

Preparation of sample stock solution

The average weight of 20 tablets was determined and was ground in a mortar. Take Tablet Powder equivalent to 1 mg of Cilnidipine and 30 mg of Irbesartan was transferred to a 100 ml volumetric flask, Add 60 ml Mobile phase, Shaken for few Minutes and make up volume with Mobile phase. The solution was filtered through Whatman filter paper no. 42.

Working Sample Preparation

Take 1 ml from standard stock solution and transferred to 10 ml volumetric flask and made up volume up to the mark with the mobile phase. Inject above Solution 20 µl for Assay Analysis.

Chromatographic conditions

A BDS hypersil C_{18} (25cm x 0.46 cm) column was used as the stationary phase. The chromatogram was run for appropriate minutes with mobile phase Phosphate Buffer (pH 4.5):

Methanol (85:15). The detection was carried out at wavelength 248 nm. The mobile phase was pumped at 1.0 ml/min. Total run time is 10 mins.

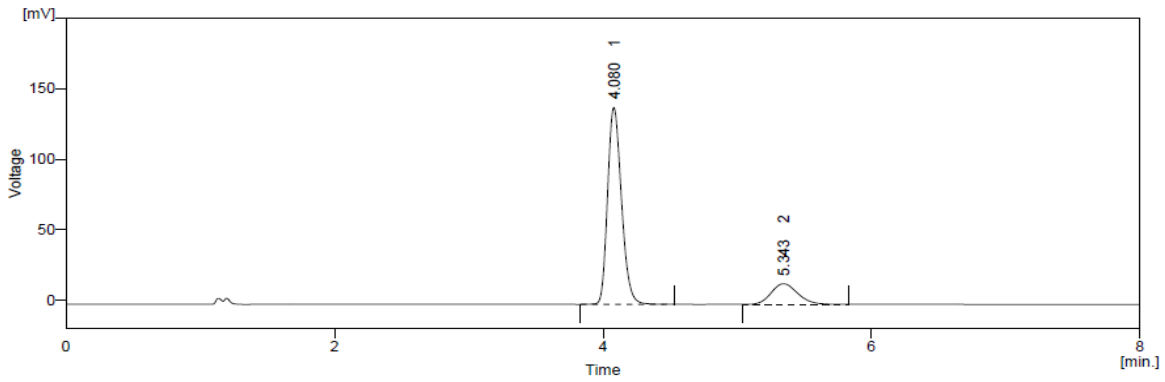

Fig. 3: Chromatogram of irbesartan and cilnidipine.

The developed Method was validated for linearity, precision, accuracy, robustness and is applied for forced degradation studies as per the ICH guidelines.

RESULTS AND DISSCUSION

Method validation

The described method include validated parameters like system suitability, linearity, accuracy, precision, robustness, LOD (limit of detection) and LOQ (limit of quantification).

System suitability

System suitability and chromatographic parameters were validated such as retention time resolution, theoretical plates and tailing factor was calculated. The results are given in table 1.

Table 1: System suitability parameters for irbesartan and cilnidipine.

System suitability Parameters	Irbesartan	Cilnidipine
Retention Time	4.080	5.343
Theoretical Plates	7180	3369
Asymmetry	1.385	1.264
Resolution	4.505	

Linearity

Linearity was evaluated by linear regression analysis and calculated by least square method. Preparing standard solutions of cilnidipine and irbesartan at different concentration levels. The calibration curve showed (Fig.4 and 5) good linearity in the range of 0.5-1.5 µg/ml for cilnidipine with Correlation co-efficient of 0.999 and 15-45 µg/ml for Irbesartan with Correlation co-efficient of 0.999. Results shown in table 2.

Table 2: Linearity data for irbesartan and cilnidipine.

Drug	Conc. (µg/ml)	Area
Irbesartan	15	510.871
	22.5	735.164
	30	1008.459
	37.5	1251.952
	45	1507.440
Cilnidipine	0.5	102.27
	0.75	147.49
	1	202.41
	1.25	251.36
	1.5	306.01

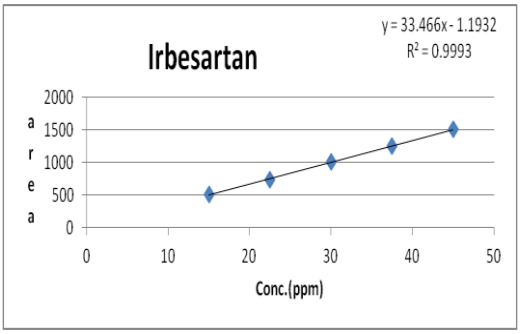

Fig. 4: Calibration curve of irbesartan.

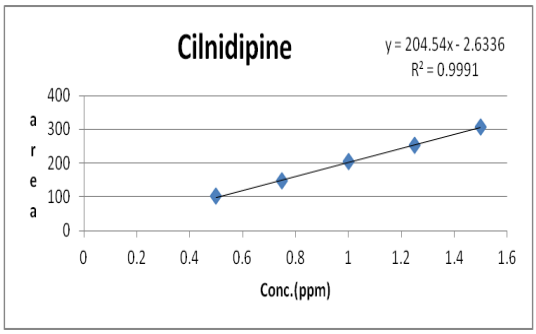

Fig.5: Calibration curve of cilnidipine.

Accuracy

Recovery study include addition of standard drug to the sample at 3 different concentration levels (80%, 100% and 120%) taking into % purity of added bulk drug samples. At each concentration, sample was injected thrice to check repeatability and from the %RSD values it was analyzed that the method was accurate as % recovery values found to be in the range of 100.312–101.296% for Irbesartan and 100.38-101.02% for Cilnidipine at three different concentrations 80%, 100%, 120%. The results are given in table 3 and 4.

Table 3: Accuracy data for irbesartan.

Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D*
80 %	15	12	12.146	101.214	100.312 ± 1.191
	15	12	12.091	100.762	
	15	12	11.875	98.962	
100 %	15	15	14.972	99.814	100.889 ± 0.948
	15	15	15.187	101.247	
	15	15	15.241	101.606	
120 %	15	18	18.134	100.747	101.296 ± 0.663
	15	18	18.366	102.032	
	15	18	18.200	101.109	

S.D - Standard deviation, Number of experiments (n) – 3*

Table 4: Accuracy data for cilnidipine.

Conc. Level (%)	Sample Amount	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
80 %	0.5	0.4	0.403	100.823	100.383 ± 0.973
	0.5	0.4	0.404	101.058	
	0.5	0.4	0.397	99.268	
100 %	0.5	0.5	0.501	100.122	100.937 ± 1.205
	0.5	0.5	0.512	102.321	
	0.5	0.5	0.502	100.367	
120 %	0.5	0.6	0.606	101.048	100.388 ± 0.594
	0.5	0.6	0.601	100.219	
	0.5	0.6	0.599	99.896	

S.D - Standard deviation, Number of experiments (n) – 3*

Precision

Repeatability

Standard solution containing cilnidipine (1 µg/ml) and irbesartan (30 µg/ml) was injected six times and areas of peaks were measured and % R.S.D was calculated. The results are given in table 5.

Table 5: Repeatability data for irbesartan and cilnidipine.

Drug	Conc (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
Irbesartan	30	1011.478 1009.442 996.977 1009.389 1007.360 1017.485	1008.689±6.710	0.665
Cilnidipine	1	203.033 202.648 200.811 202.615 202.200 204.242	202.592 ±1.118	0.552

S.D - Standard deviation, R.S.D* - Relative standard deviation, Number of experiments (n) - 6*

Intraday precision

Standard solution containing (15,30,45 µg/ml) of Irbesartan and (0.5,1,1.5 µg/ml) of Cilnidipine were analyzed three times on the same day and % R.S.D was calculated. The results are given in table 6.

Table 6: Intraday precision data for estimation of irbesartan and cilnidipine.

Drug	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
Irbesartan	15	507.460 ± 2.414	0.476
	30	1020.596 ± 4.406	0.432
	45	1510.421± 4.710	0.312
Cilnidipine	0.5	101.769 ± 0.177	0.174
	1.0	203.844 ± 1.446	0.709
	1.5	301.321 ± 3.841	1.274

Interday precision

Standard solution containing (15, 30, 45 µg/ml) of Irbesartan and (0.5, 1, 1.5 µg/ml) of Cilnidipine were analyzed three times on the different day and % R.S.D was calculated. The results are given in table 7.

Table 7: Interday precision data for estimation of irbesartan and cilnidipine.

Drug	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
Irbesartan	15	509.291 ± 4.371	0.858
	30	990.636± 12.191	1.231
	45	1516.091± 7.122	0.470
Cilnidipine	0.5	101.345 ± 1.913	1.888
	1.0	199.053 ± 2.104	1.057
	1.5	304.791 ± 0.914	0.300

Robustness

Small deliberate changes in chromatographic conditions such as change in mobile phase ratio (±2 %), change in pH (±2 units) and flow rate (±2 units) were studied to determine the robustness of the method. The results were in factor of (% RSD < 2%) the developed RP-HPLC method for the analysis of Irbesartan and Cilnidipine. The results are given in table 8 and 9.

Table 8: Robustness data for irbesartan.

Drug	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (-0.2)	Area at pH (+0.2)	Area at Mobile phase(-2)	Area at Mobile phase(+2)
	1050.843	971.091	1009.446	1036.833	1027.652	1000.359
Irbesartan	1046.626	971.116	1013.509	1039.940	1016.133	998.494
	1044.305	975.996	1019.587	1036.808	1024.531	1011.401
% R.S.D	0.316	0.290	0.503	0.174	0.582	0.695

R.S.D - Relative standard deviation, Number of experiments (n) – 3.*

Table 9: Robustness data for cilnidipine.

Drug	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (- 0.2)	Area at pH (+ 0.2)	Area at Mobile phase(-2)	Area at Mobile phase(+2)
	210.907	194.949	202.614	208.106	206.283	200.818
Cilnidipine	210.025	194.961	203.413	208.746	204.830	201.411
	206.131	190.205	202.069	205.066	205.649	203.046
% R.S.D	1.216	1.418	0.333	0.948	0.354	0.572

R.S.D - Relative standard deviation, Number of experiments (n) – 3.*

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

The LOD and LOQ were found to be 1.167 µg/ml and 3.538 µg/ml for Irbesartan and 0.046 µg/ml and 0.139 µg/ml for Cilnidipine estimated by standard formulas.

Table 10: LOD and LOQ data for irbesartan and cilnidipine.

Drug	LOD	LOQ
Irbesartan	1.167 µg/ml	3.538 µg/ml
Cilnidipine	0.046 µg/ml	0.139 µg/ml

Method development

ICH prescribed stress conditions such as acid, base, oxidative, photo, thermal degradation.[6]

Acid degradation

Acid decomposition studies were performed by transferring one ml of stock solution in to 10 ml of volumetric flask. Two ml of 0.1 N Hydrochloride solutions was added and mixed well and put for 5 hrs, Then the volume was adjusted with diluent to get 1 µg/ml for Cilnidipine and 30 µg/ml for Irbesartan. After making final solutions, it is injected into HPLC and the peak area and peak shapes were observed. Chromatogram of acid degradation on sample solution is shown below in figure 6.

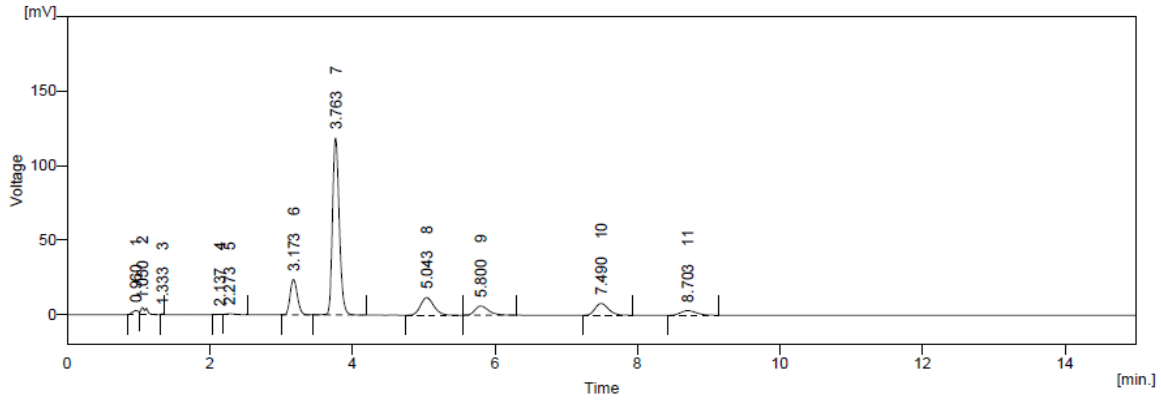

Fig. 6: Irbesartan and cilnidipine acid degradation sample.

Base degradation

Basic decomposition studies were performed by transferring one ml of stock solution in to 10 ml of volumetric flask. Two ml of 0.1 N NaOH solutions was added and mixed well and put for 4 hrs. Then the volume was adjusted with diluent to get 1 µg/ml for Cilnidipine and 30 µg/ml for Irbesartan. After making final solutions, it is injected into HPLC and the peak area and peak shapes were observed. Chromatogram of base degradation on sample solution is shown below in figure 7.

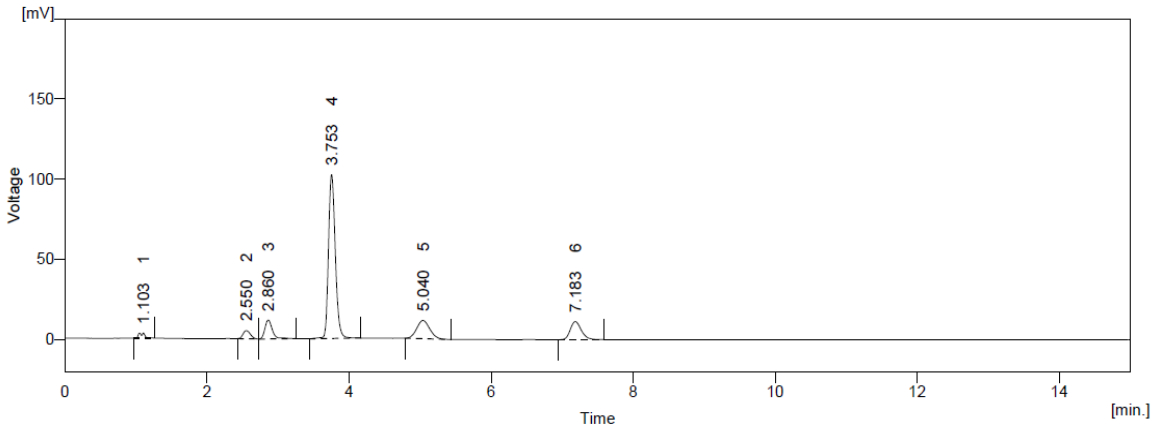

Fig. 7: Irbesartan and cilnidipine base Degradation Sample.

Oxidative degradation

Oxidative decomposition studies were performed by transferring one ml of stock solution in to 10 ml of volumetric flask. Two ml of 3% H_2O_2 solutions was added and mixed well and put for 4 hrs. Then the volume was adjusted with diluent to get 1 µg/ml for Cilnidipine and 30 µg/ml for Irbesartan. After making final solutions, it is injected into HPLC and the peak area and peak shapes were observed. Chromatogram of oxidative degradation on sample solution is shown below in figure 8.

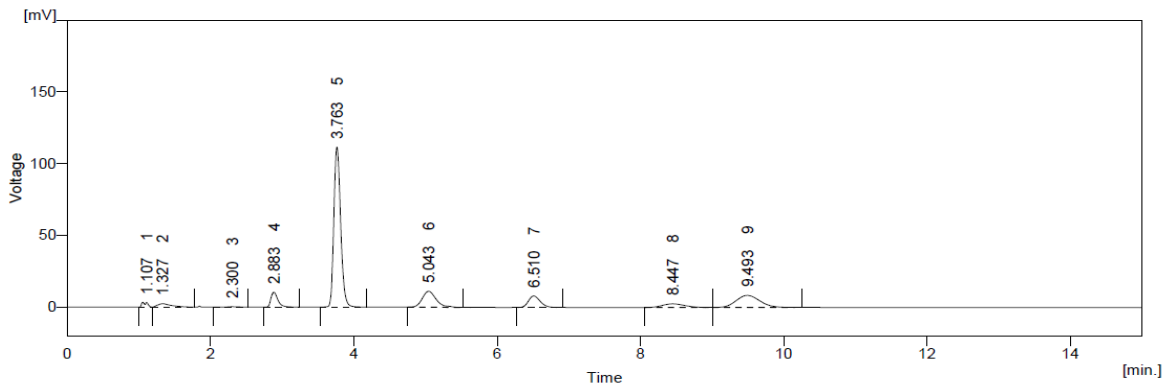

Fig. 8: Irbesartan and cilnidipine oxidation degradation sample.

Photo degradation

Photo Degradation studies were performed by transferring one ml of stock solution in to 10 ml of volumetric flask. The volumetric flask was kept in UV Chamber for 12 hrs. Then the volume was adjusted with diluent to get 1 µg/ml for Cilnidipine and 30 µg/ml for Irbesartan. After making final solutions it is injected into HPLC and the peak area and peak shapes were observed. Chromatogram of photo degradation on sample solution is shown below in figure 9.

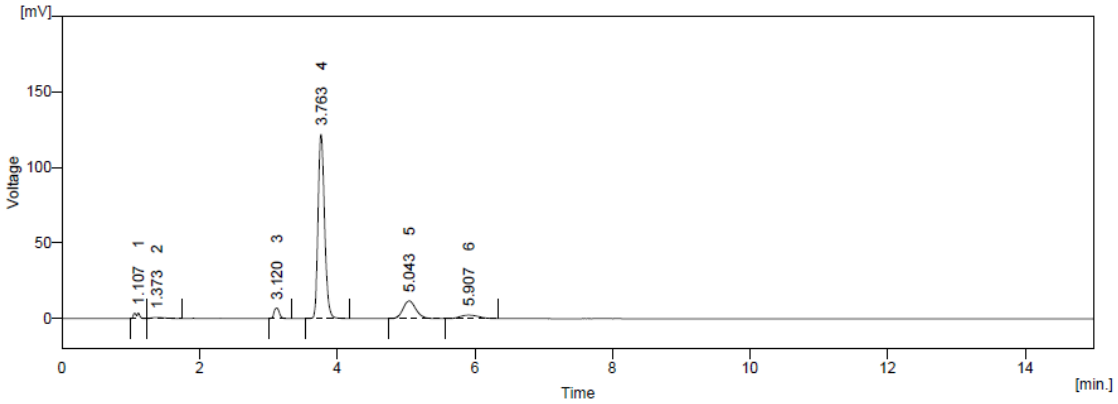

Fig. 9: Irbesartan and cilnidipine photo degradation sample.

Thermal degradation

Thermal degradation studies were performed by transferring one ml of stock solution was transferred in to 10 ml of volumetric flask. The volumetric flask was stored in oven at 70°C for 5 hrs. Then the volume was adjusted with diluent to get 1 µg/ml for Cilnidipine and 30 µg/ml for Irbesartan. After making final solutions, it is injected into HPLC and the peak area and peak shapes were observed. Chromatogram of thermal degradation on sample solution is shown below in figure 10.

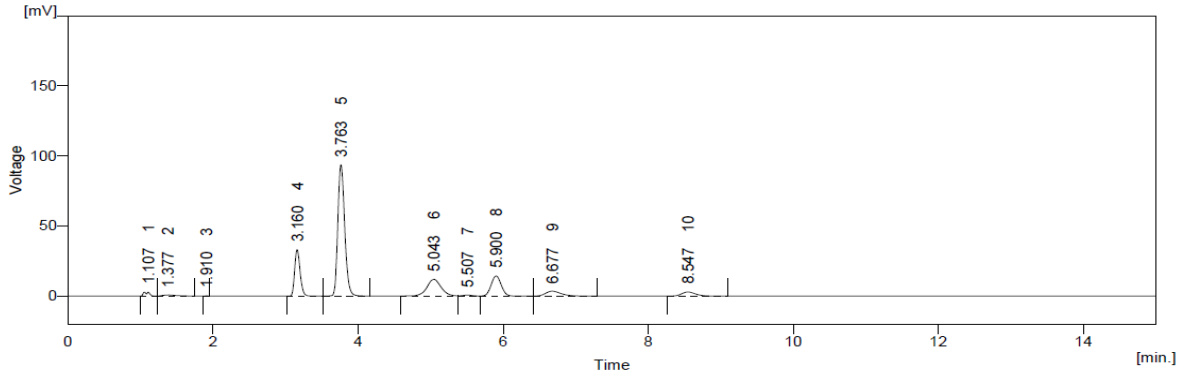

Fig. 10: Irbesartan and cilnidipine thermal degradation sample.

Table 11: Stability data for cilnidipine and irbesartan.

Cilnidipine standard area for stability - 188.104				
Parameter	Standard		Sample	
	Area	% Degradation	Area	% Degradation
Acid	150.515	19.983	153.131	18.592
Base	155.049	17.573	145.500	22.649
Thermal	158.013	15.997	163.108	13.288
Oxidation	152.928	18.700	149.068	20.752
Photo	152.282	19.044	153.976	18.143
Irbesartan standard area for stability - 916.253				
Parameter	Standard		Sample	
	Area	% Degradation	Area	% Degradation
Acid	798.943	12.803	787.870	14.012
Base	662.278	27.719	682.565	25.505
Thermal	658.708	28.108	649.163	29.150
Oxidation	749.528	18.196	738.145	19.439
Photo	815.743	10.970	806.221	12.009

Cilnidipine and Irbesartan undergoes significant degradation in acid, base, photo, thermal and oxidation. Hence, a method of the analysis of Cilnidipine and Irbesartan in tablet dosage form shows that the degradation product doesn't interfere with the analytical determination. Hence, the proposed analytical method is also useful for the determination of Cilnidipine and Irbesartan stability in a sample of the pharmaceutical dosage form.

CONCLUSION

Stability indicating RP-HPLC methods have been developed and validated for the determination of Cilnidipine and Irbesartan in tablet dosage form. The methods are found to be specific as there was no interference of any co-eluting impurities after stress degradation study. The proposed method is found to be simple, accurate, precise and rapid. Hence, it can be used successfully for the routine analysis of Cilnidipine and Irbesartan in pharmaceutical dosage forms.

ACKNOWLEDGEMENT

The authors are thankful to Rivan Pharmaceutical Ltd. for providing all the facilities to complete the research work. Special thanks to Yash Pharma, Ahmedabad, India for providing the gift sample of Cilnidipine and Irbesartan.

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