Available online on 15.07.2017 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics
Open Access to Pharmaceutical and Medical Research

Open Access Research Article

METHOD DEVELOPMENT AND VALIDATION OF IRBESARTAN CHLORTHALIDONE AND CILNIDIPINE IN THEIR COMBINED TABLET DOSAGE FORM BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Rishabh K Dagariya, Rakesh K Jat,

Institute of Pharmacy, Shri Jagadish Prasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan

ABSTRACT

The present work describes a validated reverse phase high performance liquid chromatographic method for simultaneous estimation of Irbesartan chlorthalidone and cilnidipine in tablet dosage form. The quantification was carried out using C18 column (250 x 4.6mm, 5µm) and mobile phase comprised of Buffer, Acetonitrile and TEA in a proportion of 80:20:0.1 %v/v/v. The flow rate was 1.0 ml/min and the eluent was monitored at 222 nm. The selected chromatographic conditions were found to effectively separate Irbesartan Chlorthalidone and cilnidipine were 3.807 min, 4.667 min, and 6.887 min respectively. Linearity was found to be in the range of 30-90 µg/ml, 1.25-3.75 µg/ml and 1-3 µg/ml for Irbesartan Chlorthalidone and cilnidipine respectively. The percentage recoveries of all the drugs were found to be 99.27-99.81%, 99.57-99.99% and 99.22-99.44% for Irbesartan, chlorthalidone, and cilnidipine. The proposed method was found to be fast, accurate, precise, and reproducible and can be used for simultaneous estimation of these drugs in a tablet.

Keywords: Irbesartan, chlorthalidone and cilnidipine, Reversed-phase HPLC.

Article Info: Received 28 May, 2017; Review Completed 11 July, 2017; Accepted 12 July, 2017; Available online 15 July, 2017

Cite this article as:

Dagariya RK, Jat RK, Method development and validation of Irbesartan chlorthalidone and Cilnidipine in their combined tablet dosage form by high performance liquid chromatography, Journal of Drug Delivery and Therapeutics. 2017; 7(4):88-96

DOI: http://dx.doi.org/10.22270/jddt.v7i4.1471

***Address for Correspondence**

Rishabh K Dagariya, Institute of Pharmacy, Shri Jagadish Prasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan, India Email: rishabhdagariya@gmail.com

INTRODUCTION

Irbesartan is an angiotensin converting enzyme blocker As with all angiotensin II receptor antagonists, irbesartan is indicated for the treatment of hypertension. It may also delay progression of diabetic nephropathy and is also indicated for the reduction of renal disease progression in patients with type 2 diabetes, hypertension and microalbuminuria (>30 mg/24 hours) or proteinuria (>900 mg/24 hours)

Chlorthalidone is a diuretic drug used to treat hypertension, Compared with other medications of the thiazide class, chlortalidone has the longest duration of action but a similar diuretic effect at maximal therapeutic doses.Chlorthalidone of (RS)-2-Chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1H-isoindol-1-yl)benzene-1-sulfonamide, represents the class of Chlortalidone is a diuretic drug used to treat hypertension Chlortalidone has the longest duration of action but a similar diuretic effect at maximal therapeutic doses., used as an Antihypertensive agent.[1-2] Chlorthalidone inhibits sodium ion transport across the renal tubular epithelium in the cortical diluting segment of the ascending limb of the loop of Henle. By increasing the delivery of sodium to the distal renal tubule, Chlorthalidone indirectly increases potassium excretion via the sodium-potassium exchange mechanism. The individual and combination with other drugs determination of Chlorthalidone has been carried out by UV spectrophotometer [3], RP-HPLC [4-6] in bulk and solid dosage forms. Cilnidipine of O3-(2-

methoxyethyl)O5-[(E)-3-phenylprop-2-enyl]2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine- 3,5-dicarboxylate belongs to Anti hypertensive.[7-9] Literature survey revealed that few analytical methods have been reported for the estimation of Cilnidipine included RP-HPLC[10-11] UV spectrophotometer.[12-13].Cilnidipine is a dual L-/N-type calcium channel protein inhibitor and blocker. Cilnidipine has displayed renal and vascular protective effects and improved baroreflex sensitivity in patients with hypertension. The present work describes a validated reverse phase HPLC method for simultaneous determination of these drugs in tablets.

MATERIALS AND METHODS

Reagents and Chemicals: Irbesartan, chlorthalidone and cilnidipine were received as a gift sample from Molecule laboratory, Acetonitrile of HPLC grade, Triethyl amine of HPLC grade, Water of HPLC grade.

Instruments and Chromatographic Conditions: LC-10AT HPLC system was used for method development, degradation studies and validation. Data acquisition was performed on Spinchrom HPLC software. The separation were achieved on C18 (250 × 4.6 mm, 5µm) column. The column was maintained at room temperature and the eluent was monitored at 222 nm using UV detector. The mixture of Potassium dihydrogen phosphate buffer 0.05M (pH 3.5): Acetonitrile: TEA (80:20:0.1 v/v) at a flow rate of 1.0 ml/min was used as a mobile phase. The injection volume was 20µl.

Preparation of standard solutions:

Irbesartan standard stock solution: (600 µg/mL)

A 60 mg of Irbesartan was weighed and transferred to a 100 mL volumetric flask. Volume was made up to the mark with methanol.

Cilnidipine standard stock solution: (20 µg/mL)

A 20 mg of Cilnidipine was weighed and transferred to a 100 mL volumetric flask. Volume was made up to the mark with methanol. Take 10ml from this solution and transferred to 100 mL volumetric flask and volume made up to the mark by mobile phase.

Chlorthalidone standard stock solution: (25 µg/mL)

A 25 mg of Chlorthalidone was weighed and transferred to a 100 mL volumetric flask. Volume was made up to the mark with methanol. Take 10ml from this solution and transferred to 100 mL volumetric flask and volume made up to the mark by mobile phase.

Preparation of standard solution of mixtures of Irbesartan (60 µg/mL),Cilnidipine (2 µg/mL) and Chlorthalidone (2.5 µg/mL)

Take 1 mL from the Irbesartan stock solution, 1mL from Cilnidipine stock solution and 1mL from Chlorthalidone stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by mobile phase which was used in particular trials.

Preparation of Mobile phase:

Potassium dihydrogen phosphate buffer 0.05M (pH 3.5): Acetonitrile: TEA (80:20:0.1 v/v)

Preparation of sample solution:

Take Crushed Tablet powder equivalent to 60 mg of Irbesartan, 2.5 mg Chlorthalidone and 2 mg of Cilnidipine was transferred to a 100 ml volumetric flask, and made up volume up to the mark with mobile phase. The solution was filtered through whatman filter paper no. 42 and first few drops of filtrate were discarded. 10 ml of this solution was diluted to 100 ml with mobile phase. The solution was injected 20 µl. The areas of resulting peak were measured at 222 nm.

Validation of RP-HPLC method

1 Linearity

The linearity for Irbesartan, Cilnidipine and Chlorthalidone were assessed by analysis of combined standard solution in range of 30-90 µg/ml, 1-3 µg/ml and 1.25-3.75 µg/ml respectively,

5,7.5,10,12.5,15 ml solutions were pipette out from the Stock solution of Irbesartan(60 µg/ml), Cilnidipine (2 µg/ml) and Chlorthalidone (2.5 µg/ml) and transfer to 100 ml volumetric flask and make up with mobile phase to obtain 30,45,60,75 and 90 µg/ml, 1,1.5,2,2.5 and 3 µg/ml and 1.25,1.875,2.5,3.125 and 3.75 µg/ml for Irbesartan, Cilnidipine and Chlorthalidone respectively

In term of slope, intercept and correlation co-efficient value. The graph of peak area obtained verses respective concentration was plotted.

2 Precision

Results should be expressed as Relative standard deviation (RSD) or coefficient of variance.

A. Repeatability

Standard solution containing Irbesartan (60 µg/ml), Cilnidipine (2 µg/ml) and Chlorthalidone (2.5 µg/ml) was injected six times and areas of peaks were measured and % R.S.D. was calculated.

B. Intra-day precision

Standard solution containing (30,60,90 µg/ml) of Irbesartan and (1,2,3 µg/ml) of Cilnidipine and (1.25,2.5,3.75 µg/ml) of Chlorthalidone were analyzed three times on the same day and % R.S.D was calculated.

C. Inter-day precision

Standard solution containing (30,60,90 µg/ml) of Irbesartan and (1,2,3 µg/ml) of Cilnidipine and (1.25,2.5,3.75 µg/ml) of Chlorthalidone were analyzed three times on the different day and % R.S.D was calculated.

3 Accuracy

For Irbesartan

30 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 222 nm. The amount of

Irbesartan was calculated at each level and % recoveries were computed.

For Cilnidipine

1 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 222 nm. The amount of Cilnidipine was calculated at each level and % recoveries were computed.

For Chlorthalidone

1.25 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 222 nm. The amount of Chlorthalidone was calculated at each level and % recoveries were computed.

4 LOD and LOQ

The LOD was estimated from the set of 3 calibration curves used to determination method linearity. The LOD may be calculated as,

LOD = 3.3 × (SD/Slope)

Where,

SD= Standard deviation of Y-intercepts of 3 calibration curves.

Slope = Mean slope of the 3 calibration curves.

The LOQ was estimated from the set of 3 calibration curves used to determine method linearity. The LOQ may be calculated as,

LOQ = 10 × (SD/Slope)

Where,

SD = Standard deviation of Y-intercepts of 3 calibration curves.

Slope = Mean slope of the 3 calibration curves.

5 Robustness

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

1. Flow rate of mobile phase was changed (± 0.2 ml/min) 0.8 ml/min and 1.2 ml/min.

2. pH of Mobile phase was changed (± 0.2) 3.3 and 3.5.

3. Ratio of Mobile phase was changed (±2) Buffer: Acetonitrile (82:18) and Buffer: Acetonitrile (78:22)

RESULT AND DISCUSSION

Specificity:

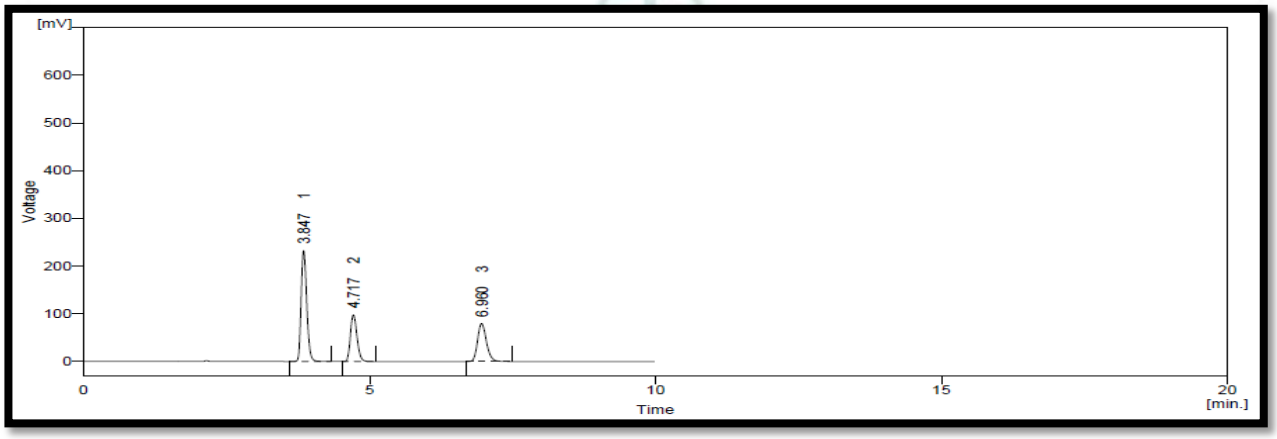

Figure 1: Chromatogram of Irbesartan, Chlorthalidone and Cilnidipine standard

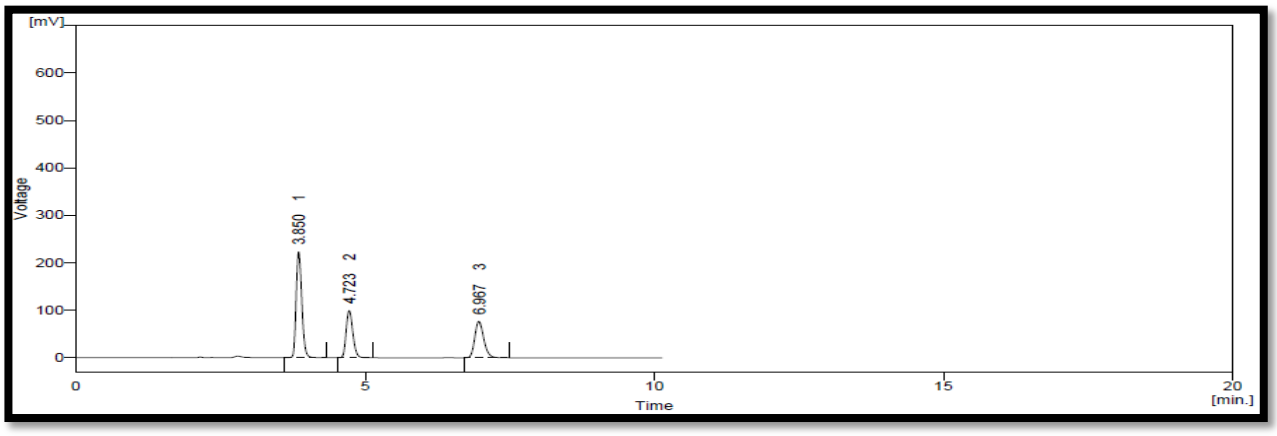

Figure 2: Chromatogram of Irbesartan, Chlorthalidone and Cilnidipine sample

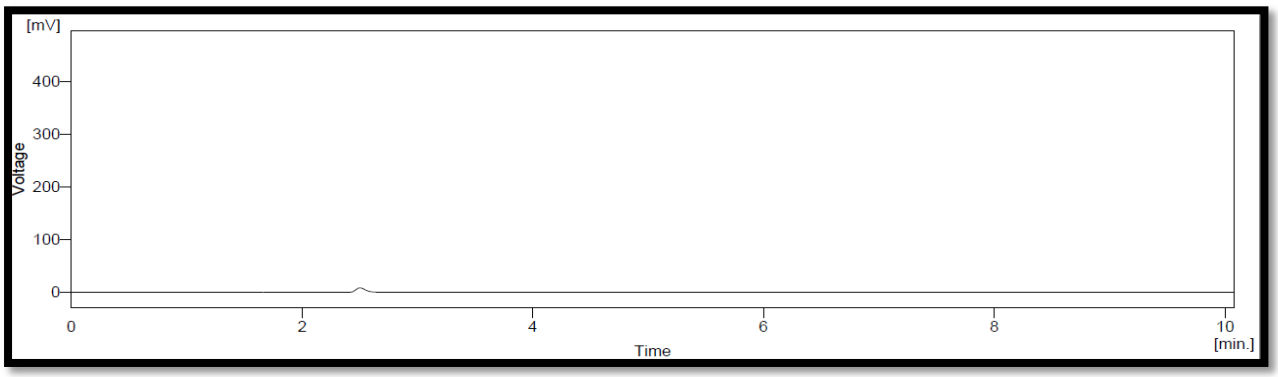

Figure 3: Chromatogram of Irbesartan, Chlorthalidone and Cilnidipine Blank

The Chromatograms of Irbesartan, Chlorthalidone and Cilnidipine standards and Irbesartan, Chlorthalidone and Cilnidipine sample show no interference with the Chromatogram of Irbesartan, Chlorthalidone and Cilnidipine Blank, so the Developed method is Specific.

Linearity and Range

The linearity for Irbesartan, Cilnidipine and Chlorthalidone were assessed by analysis of combined standard solution in range of 30-90 µg/ml,1-3 µg/ml and 1.25-3.75 µg/ml respectively. Correlation co-efficient for calibration curve Irbesartan, Cilnidipine and Chlorthalidone was found to be 0.999 for all of three.

The regression line equation for Irbesartan, Cilnidipine and Chlorthalidone are as following:

For Irbesartan:

y = 26.20x - 10.80 and

For Chlorthalidone:

y = 311.1x - 5.764 and

For Cilnidipine

y = 425.9x - 5.533

Table 1: Linearity data for Irbesartan

Sr.No	Concentration (µg/ml)	Area
1	30	776.733
2	45	1162.735
3	60	1570.086
4	75	1949.034
5	90	2348.984

Table 2: Linearity data for Chlorthalidone

Sr. No	Concentration (µg/ml)	Area
1	1.25	384.037
2	1.875	574.785
3	2.5	776.051
4	3.125	963.331
5	3.75	1162.027

Table 3: Linearity data for Cilnidipine

Sr. No	Concentration (µg/ml)	Area
1	1	420.91
2	1.5	630.213
3	2	851.093
4	2.5	1056.653
5	3	1272.432

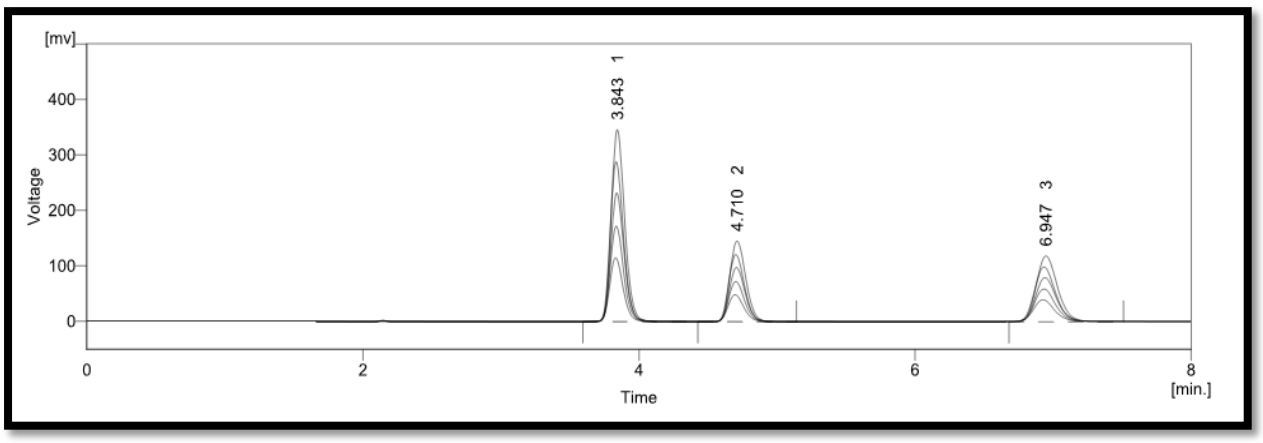

Figure 4: Overlay chromatogram of different concentrations of mixtures of Irbesartan, Cilnidipine and Chlorthalidone

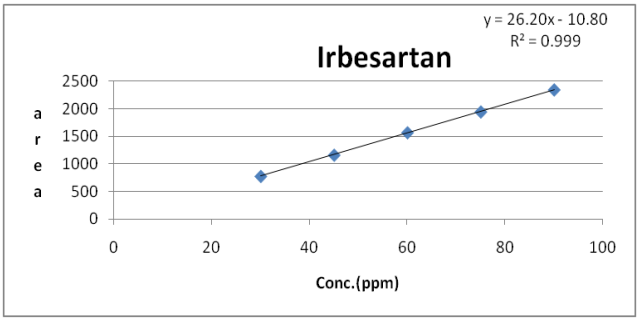

Figure 5: Calibration Curve of Irbesartan (30-90 µg/ml)

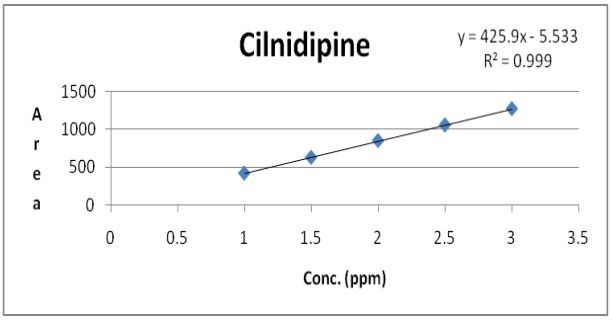

Figure 7: Calibration Curve of Cilnidipine (1-3 µg/ml)

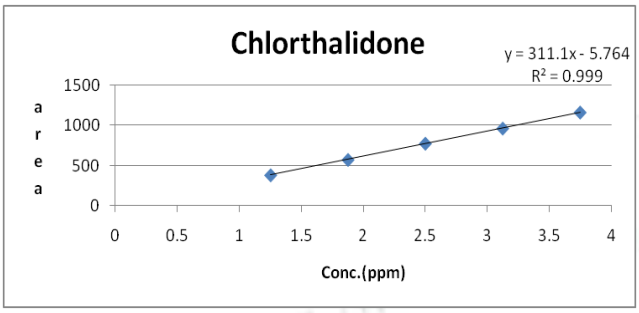

Figure 6: Calibration Curve of Chlorthalidone (1.25-3.75 µg/ml)

Precision

I. Repeatability

The data for repeatability of peak area measurement for Irbesartan(60 µg/ml), Cilnidipine (2 µg/ml) and Chlorthalidone (2.5 µg/ml), based on six measurements of same solution of Irbesartan (60 µg/ml), Cilnidipine (2 µg/ml) and Chlorthalidone (2.5 µg/ml) are depicted in table. The % RSD for Irbesartan, Chlorthalidone and Cilnidipine were found to be 0.271, 0.249 and 0.154 respectively.

Table 4: Repeatability data for Irbesartan

Irbesartan				
Sr No.	Conc (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
1.	60	1565.283	1568.45±4.25	0.271
		1563.976		
		1566.882		
		1570.032		
		1568.622		
		1575.875		

Table 5: Repeatability data for Chlorthalidone

Chlorthalidone				
Sr No.	Conc (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
1.	2.5	773.736	775.93 ±1.93	0.249
		775.27		
		774.495		
		776.049		
		776.831		
		779.169		

Table 6: Repeatability data for Cilnidipine

Cilnidipine				
Sr No.	Conc (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
1.	2	848.569	850.45 ±1.30	1.153
		850.25		
		849.382		
		851.102		

		851.945		
		851.473		

II. Intraday precision

The data for intraday precision for Irbesartan, Cilnidipine and Chlorthalidone is shown in table 6.10. The % R.S.D for Intraday precision was found to be 0.148-0.554 for Irbesartan and 0.198-0.312 for Chlorthalidone and 0.568-1.062 for Cilnidipine.

Table 7: Intraday precision data for estimation of Irbesartan

	Irbesartan		
SR. NO.	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	30	769.78 ± 2.76	0.359
2	60	1558.92± 2.31	0.148
3	90	3498.33± 19.38	0.554

Table 8: Intraday precision data for estimation of Chlorthalidone

	Chlorthalidone		
SR. NO.	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	1.25	381.73 ± 0.76	0.198
2	2.5	771.40± 1.53	0.200
3	3.75	1734.56 ± 5.42	0.312

Table 9: Intraday precision data for estimation of Cilnidipine

	Cilnidipine		
SR. NO.	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	1	416.24 ± 3.80	0.914
2	2	844.11± 4.80	0.568
3	3	1886.32 ± 20.03	1.062

III. Interday precision

The data for intraday precision Irbesartan, Cilnidipine and Chlorthalidone is shown in table 5.11. The % RSD for interday precision was found to be 0.236-0.978 for Irbesartan and 0.198-0.805 for Chlorthalidone and 0.384-1.029 for Cilnidipine

.. Table 10: Interday precision data for estimation of Irbesartan

	Irbesartan		
SR. NO.	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	30	769.55 ± 1.82	0.236
2	60	1544.75± 15.12	0.979
3	90	3502.97± 8.83	0.252

Table 11: Interday precision data for estimation of Chlorthalidone

	Chlorthalidone		
SR. NO.	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	1.25	381.36 ± 0.76	0.199
2	2.5	768.07± 5.82	0.757
3	3.75	1723.19 ± 13.88	0.806

Table 12: Interday precision data for estimation of Cilnidipine

	Cilnidipine		
SR. NO.	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	1	416.99 ± 1.60	0.385
2	2	843.80± 4.56	0.540
3	3	1886.90 ± 19.43	1.29

Accuracy:

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. The results are shown in table 6.12 and 6.13. Percentage recovery for Irbesartan was 99.27-99.81 % and Chlorthalidone, it was found to be in range of 99.57-99.99% and for Cilnidipine 99.22-99.44%

Table 13: Recovery data for Irbesartan

SR. NO.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	30	24	23.560	98.17	99.81 ± 1.48
2		30	24	24.245	101.02	
3		30	24	24.059	100.24	
4	100 %	30	30	29.406	98.02	99.27 ± 1.09
5		30	30	30.027	100.09	
6		30	30	29.909	99.70	
7	120 %	30	36	35.941	99.83	99.57 ± 0.38
8		30	36	35.687	99.13	
9		30	36	35.910	99.75	

Table 14: Recovery data for Chlorthalidone

SR. NO.	Conc. Level (%)	Sample Amount	Amount Added	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	1.25	1	0.988	98.77	99.99 ± 1.12
2		1.25	1	1.010	100.99	
3		1.25	1	1.002	100.22	
4	100 %	1.25	1.25	1.237	98.97	99.57 ± 0.56
5		1.25	1.25	1.251	100.07	
6		1.25	1.25	1.246	99.67	
7	120 %	1.25	1.5	1.504	100.28	99.70 ± 0.59
8		1.25	1.5	1.487	99.10	
9		1.25	1.5	1.496	99.72	

Table 15: Recovery data for Cilnidipine

SR. NO.	Conc. Level (%)	Sample Amount	Amount Added	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	1	0.8	0.791	98.88	99.41 ± 0.80
2		1	0.8	0.792	99.03	
3		1	0.8	0.803	100.33	
4	100 %	1	1	0.991	99.08	99.22 ± 0.51
5		1	1	0.988	98.79	
6		1	1	0.998	99.79	
7	120 %	1	1.2	1.205	100.41	99.44 ± 1.22

8		1	1.2	1.177	98.08	
9		1	1.2	1.198	99.84	

LOD and LOQ

Calibration curve was repeated for five times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated as follows:

LOD = 3.3 * SD/slope of calibration curve

LOQ = 10 * SD/slope of calibration curve

Where, SD = Standard deviation of intercepts

Table 16: Limit of Detection data for Irbesartan and Chlorthalidone and Cilnidipine

Irbesartan	Chlorthalidone	Cilnidipine
LOD = 3.3 x (SD / Slope) = 3.3 x (26.20/10.80) = 0.862 µg/ml	LOD = 3.3 x (SD / Slope) = 3.3 x (311.1/5.764) = 0.036 µg/ml	LOD = 3.3 x (SD / Slope) = 3.3 x (425.9/5.533) = 0.028 µg/ml

Table 17: Limit of Quantitation data or Irbesartan and Chlorthalidone and Cilnidipine

Irbesartan	Chlorthalidone	Cilnidipine
LOQ = 10 x (SD / Slope) = 10 x (26.20/10.80) = 2.612 µg/ml	LOQ = 10 x (SD / Slope) = 10 x (311.1/5.764) = 0.111 µg/ml	LOQ = 10 x (SD / Slope) = 10 x (425.9/5.533) = 0.085 µg/ml

Robustness:

The effect of changes was found to be within the acceptance criteria as shown in table 6.16 and table 6.17. The % RSD should be less than 2%.

Table 18: Robustness data for Irbesartan

S N.	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)
1	1611.009	1521.656	1597.439	1488.475	1595.100	1520.022
2	1629.813	1533.886	1612.474	1502.462	1614.094	1533.886
3	1639.308	1538.695	1623.572	1508.516	1618.700	1544.878
% R.S.D	0.885	0.574	0.814	0.685	0.777	0.813

Table 19: Robustness data for Chlorthalidone

S N.	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)
1	800.912	753.540	791.556	738.010	793.148	753.540
2	805.609	758.188	801.985	742.655	797.837	758.188
3	810.316	760.536	802.535	747.293	799.419	763.610
% R.S.D	0.584	0.470	0.775	0.625	0.409	0.665

Table 20: Robustness data for Cilnidipine

S. N.	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)
1	878.345	826.448	868.092	809.448	869.840	826.448
2	871.187	819.804	871.991	805.584	872.866	821.574
3	888.635	834.112	880.104	819.590	877.463	837.464
% R.S.D	0.997	0.866	0.702	0.891	0.439	0.983

Analysis of marketed formulation by developed method.

Applicability of the proposed method was tested by analyzing the commercially available Tablet formulation Clindasartan-CH. The results are shown in following table.

Table 21: Showing the analysis of marketed formulation by developed method

Tablet	Clindasartan-CH		
mg/Tablet powder	Irbesartan (150 mg)	Chlorthalidone (6.25 mg)	Cilnidipine (5 mg)
Assay (% of label claim*) Mean ± S. D.	100.05±0.544	101.45±0.313	100.92±0.277

The assay results were comparable to labeled value of each drug in Tablet dosage form. These results indicate that the developed method is accurate, precise, simple and rapid. It can be used in the routine quality control of dosage form in industries.

CONCLUSION

From the above discussion it can be concluded that the proposed method is precise, accurate and stability indicating. Results are in good agreement with label claim which indicates there is no interference of excipients. Therefore the proposed method can be used for routine analysis of Irbesartan Chlorthalidone and Cilnidipine in combined tablet formulation.

ACKNOWLEDGEMENT

The authors are also thankful to Institute of Pharmacy, Shri Jagadish Prasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan for providing necessary equipment, facility & chemicals to complete research work.

REFERENCES

1. "Drug profile for Chlorthalidone", Sep 2015, http://www.drugbank.ca/drugs/DB00310
2. "Drug profile for Chlorthalidone", Sep 2015,http://en.wikipedia.org/wiki/ Chlorthalidone
3. "Drug profile for Cilnidipine", Sep 2015, en.wikipedia.org/wiki/Cilnidipine.
4. "Drug profile for Cilnidipine", Sep 2015, www.drugbank.ca/drugs/DB03614
5. Niraimathi V, Jerad SA, Kumar SI. Uv Spectrophotometric Methods For The Estimation OF Chlorthalidone In Bulk and Oral Dosage Form. Indo American J. Pharm,2013; 3(9):7160-7167.
6. Rang and Del's Pharmacology; 6th Edn; Churchil levingstone Publishers (P) Ltd, New York, 2004, pp 277-285.
7. Dong WM. Modern HPLC for Practicing Scientists; A Wiley- Inter science publication, USA, 2006, pp 1-9.
8. Kazakevich Y and LoBrutto R. HPLC for pharmaceutical Scientists; A John Wiley and sons, 2007, pp 1-6.
9. Mhaske RA, Sahsrabhdhe S, mhaske AA, "RP-HPLC Method For Simultataneous Determination of Irbesartan, Losartan, Hydro-Chlorothiazide And Chlorthalidone–Application To Commercially Available Drug Products" Int. j. pharm. Sci. and res., 2014; 1116-1123
10. Gandla K, Rao JS, Kumar JR, "New Stability Indicating Validated RP-HPLC Method for Simultaneous Estimation of Irbesartan and Atorvastatin in Combined Tablet Dosage Forms" Ame. J. pharm. Tech. res., 2015; 5(4):380-390
11. Gandla K, Suthakaran R, Kandoji P, "Development and Validation of RP-HPLC Method for Simultaneous Estimation of Irbesartan and Simvastatin in Tablet Dosage Form" Ame. J. pharm. And health res., 2014; 2(10):199-205
12. Patel S, Patel D" Simultaneous Determination of Metoprolol Succinate And Chlorthalidone By UV Spectrophotometric Method." Pharmagene. 2013; 1(3):39-43
13. Naazneen S, Sridevi A," Stability-Indicating RP-HPLC Method For The Simultaneous Estimation of Azilsartan Medoxomil And Chlorthalidone In Solid Dosage Forms. " Int. J. of Pharm. and Pharm. Sci. 2014; 6(6):236-243
14. Saravanan V., Revathi R., Meera N. Method development and validation for the simultaneous estimation of lycopene and ubidecarenone by RP-HPLC in combined pharmaceutical dosage form. Journal of Drug Delivery and Therapeutics, 2016; 6(5): 46-51.
15. Lee KH, Park HJ, Shin CY, Myung CS "Pharmaceutical composition comprising cilnidipine and telmisartan", European Patents, PCT/KR2009/002539, 2010