Chemistry Research Journal, 2023, 8(3):100-112
Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

Method Development and Validation for Simultaneous Estimation of Related Impurities of Cilnidipine and Chlorthalidone in Tablet Dosage Form by RP-HPLC

Keshav Vashistha*, Dr. Hamendra Pratap Singh

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Bhupal Nobles' University, Udaipur (Rajasthan) 313001

Abstract

A novel HPLC method was developed and validated for the estimation of related impurities of cilnidipine and chlorthalidone in pharmaceutical formulations. The chromatographic separation was carried out by isocratic elution using an Hypersil BDS C18 column (250×4.6mm; 5µ). The mobile phase was composed of phosphate buffer at a pH of 4.0 and acetonitrile in the ratio of 80:20 (V/V) at a flow rate of 1.0 mL/min. The eluents were detected and quantified at a UV detection wavelength of 225 nm. Calibration curves of all analytes in the range of 1-15µg/mL showed a good correlation linearly ($r \geq 0.99$) with recovery rate of more than 98% for each analyte. The percentage RSD in intraday, interday precision and ruggedness were found to be less than 5. Small variations in the developed conditions like mobile phase ratio, flow rate, pH and UV wavelength do not influence the results. The detailed quantitative results of this study show that this method is simple, quick, precise, accurate, sensitive, cost-effective and robust. Thus, the method development and validation result confirm that this method be successfully applied for determination and quantification of impurities for the routine quality control analysis in pharmaceutical dosage forms.

Keywords: Chlorthalidone, Cilnidipine, Related Substances, Impurity, Rp-HPLC

1. Introduction

Chlorthalidone is chemically 2-Chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1*H*-isoindol-1-yl)benzene-1-sulfonamide and it is in the sulfamoyl benzamide class. As it lacks the benzothiadiazine structure of the thiazide-type diuretics, it is called a thiazide-like diuretic [1]. Chlortalidone is freely soluble in dimethylacetamide (DMA), dimethylformamide (DMF), dimethylsulfoxide (DMSO), and methanol; it is also soluble in warm ethanol [2].

Cilnidipine is chemically 3-(*E*)-3-Phenyl-2-propenyl 5-2-methoxyethyl 2,6-dimethyl-4-(*m*-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate and it is a calcium channel blocker, calcium antagonist accompanied with L-type and N-type calcium channel blocking functions [3].

Literatures available on various UV methods are reported in the literature for the estimation of Cilnidipine and Chlorthalidone individually and in-combination with other drugs [4] and on various RP-HPLC methods are reported [5-7]. According to literature survey there is no official method for the simultaneous estimation of related impurities for Chlorthalidone and Cilnidipine by RP-HPLC in pharmaceutical dosage forms. In this study, an HPLC method

was optimized and validated for simultaneous estimation and validation of related impurities in pharmaceutical dosage forms in accordance with the ICH guidelines [8, 9].

2. Materials and Methods

Chemicals & Reagents used: Cilnidipine, Chlorthalidone, Water and Methanol for HPLC, Acetonitrile for HPLC.

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 4.0): Acetonitrile (80:20 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

Chlorthalidone standard stock solution: (1000 µg/mL)

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

Cilnidipine standard stock solution: (1000 µg/mL)

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

Impurity-1 Standard stock solution: (1000 µg/mL)

A 10 mg of Impurity-1 was weighed and transferred to a 10 mL volumetric flask. Volume was made up to the mark with methanol.

Impurity-2 Standard stock solution: (1000 µg/mL)

A 10 mg of Impurity-2 was weighed and transferred to a 10 mL volumetric flask. Volume was made up to the mark with methanol.

Preparation of standard solution of mixtures of Chlorthalidone and Cilnidipine (10 µg/mL)

Take 1 mL from the Chlorthalidone 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 Combine standard solution of Impurity-1 and Impurity-2 (10 µg/mL)

Take 1 mL from the Impurity-1 stock solution and 1mL from the Impurity-2 stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by mobile phase.

Preparation of Mobile phase

Potassium dihydrogen phosphate buffer 0.05M (pH 4.0): Acetonitrile (80:20: v/v)

Preparation of sample solution (Chlorthalidone 1000 µg/mL)

Weigh and powdered 10 tablets. Take tablet powder equivalent to 100mg of Chlorthalidone into a 100ml volumetric flask. Add 60ml mobile phase and shake for 10 minutes and sonicate for 5mins. Make up volume with mobile phase. Filter the solution with Whatman filter paper no-1 and first few drops of filtrate were discarded. The solution was injected 20 µl. The areas of resulting peak were measured at 225 nm.

3. Validation of RP-HPLC Method

1) Linearity

The linearity for Chlorthalidone, Cilnidipine, Impurity-1 and Impurity-2 were assessed by analysis of combined standard solution in range of 5-15 µg/ml respectively 0.5, 0.75, 1.0, 1.25, 1.5 ml solutions were pipette out from the Stock solution of Chlorthalidone, Cilnidipine, Impurity-1 and Impurity-2 and transfer to 100 ml volumetric flask and make up with mobile phase to obtain 5, 7.5, 10, 12.5 and 15 µg/ml, 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 Chlorthalidone, Cilnidipine, Impurity-1 and Impurity-2 (10 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 Chlorthalidone, Cilnidipine, Impurity-1 and Impurity-2 (10 µg/mL) were analyzed three times on the same day and % R.S.D was calculated.

c) Inter-day precision

Standard solution containing Chlorthalidone, Cilnidipine, Impurity-1 and Impurity-2 (10 µg/mL) were analyzed three times on the different day and % R.S.D was calculated.

3) Accuracy

For Impurity -1 and Impurity-2 (10 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 225 nm. The amount of Impurity -1 and Impurity-2 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,

$$LOD = 3.3 \times (SD/Slope)$$

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,

$$LOQ = 10 \times (SD/Slope)$$

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) 3.8 and 4.2.
3. Ratio of Mobile phase was changed (±2) Buffer: Acetonitrile (82:18) and Buffer: Acetonitrile (78:22)

4. Result and Discussion

Specificity

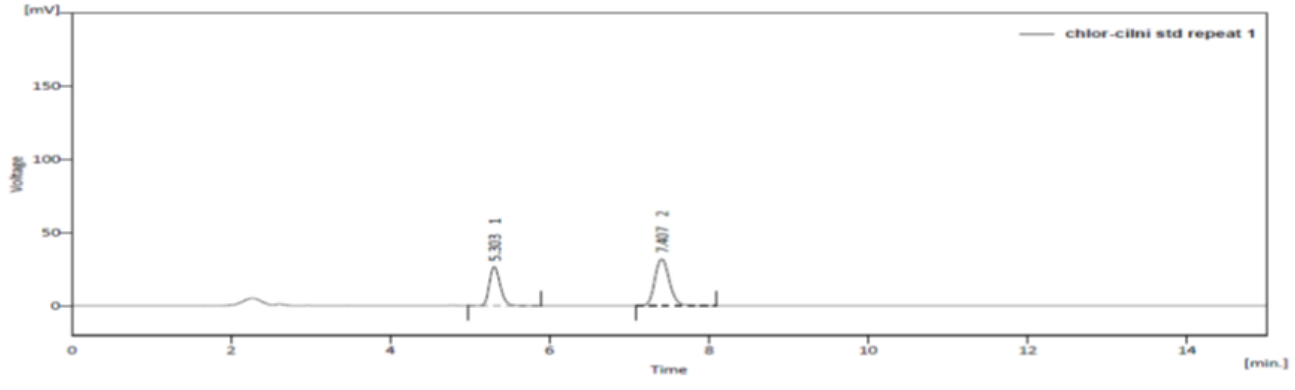

Figure 1: Chromatogram of Cilnidipine and Chlorthalidone standard

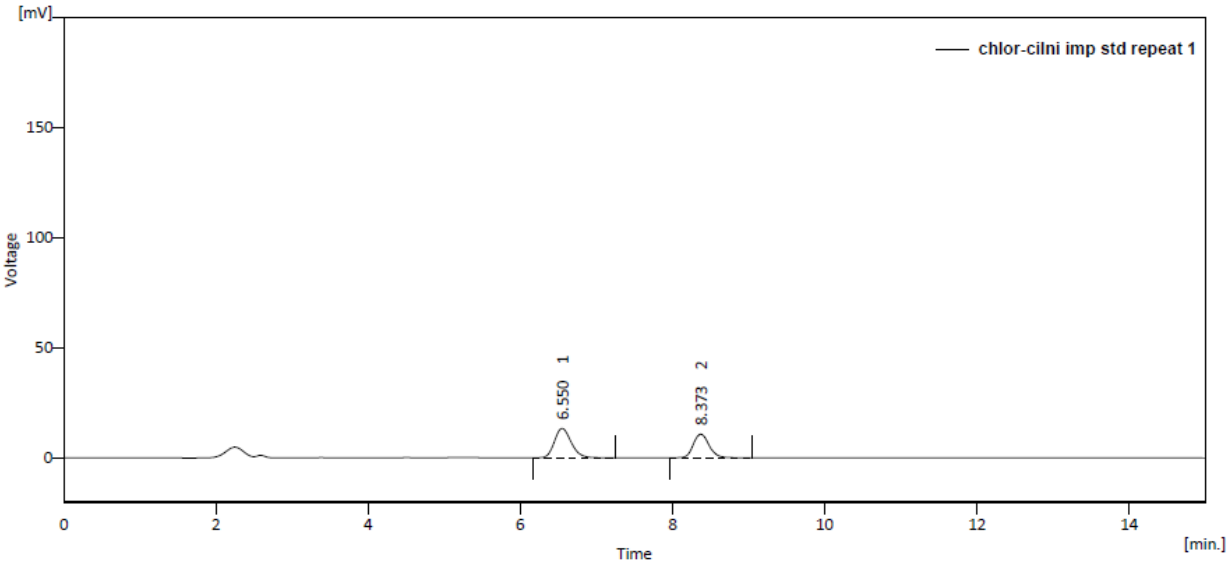

Figure 2: Chromatogram of Impurity-1 and Impurity-2 standard

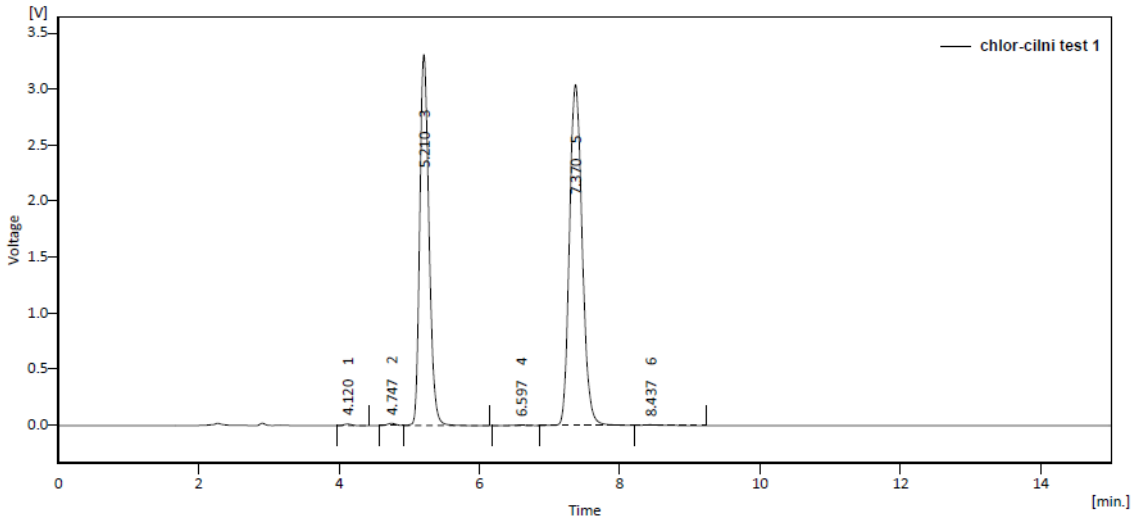

Figure 3: Chromatogram of Cilnidipine and Chlorthalidone sample in drug

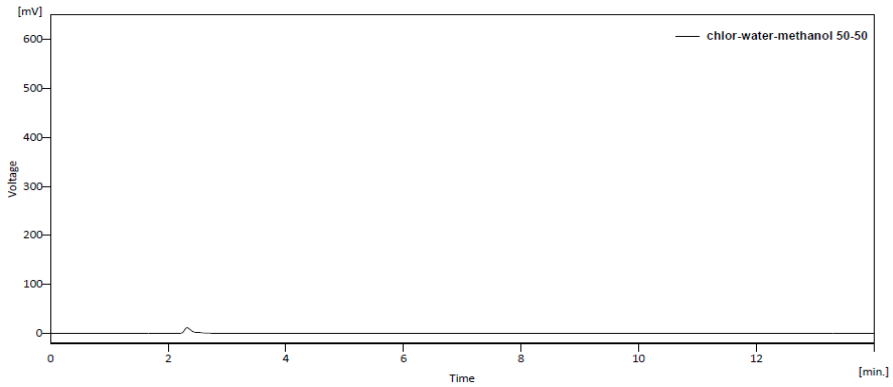

Figure 4: Chromatogram of drug Blank

The Chromatograms of Chlorthalidone, Cilnidipine, Impurity-1 and Impurity-2 standard and Chlorthalidone, Cilnidipine, Impurity-1 and Impurity-2 sample show no interference with the Chromatogram of Chlorthalidone, Cilnidipine, Impurity-1 and Impurity-2 Blank, so the developed method is Specific.

Linearity and Range

The linearity for Cilnidipine, Chlorthalidone drug, impurity-1 and impurity-2 were performed by analysis of standard solution combined in range of 5-15 µg/ml drug concentration respectively. The Correlation co-efficient value for all drug components of linearity curve was achieved as 0.99. Regression line equation for Cilnidipine drug and Chlorthalidone drug and impurity-1 and impurity-2 components are as following:

For Cilnidipine drug: **y = 44.79x + 44.3** and for Chlorthalidone drug: **y = 23.38x + 10.83 a n d** for impurity-1 **y = 19.13x + 17.60,** for impurity-2= **y = 14.59x + 12.88**

Table 1: Linearity data for Cilnidipine drug

S. No.	Concentration (µg/ml)	Peak Area
1	1	69.508
2	5	198.885
3	7.5	289.558
4	10	396.117
5	12.5	487.404
6	15	591.841

Table 2: Linearity data for Chlorthalidone drug

S. No.	Concentration (µg/ml)	Peak Area
1	1	41.351
2	5	122.982
3	7.5	179.267
4	10	245.324
5	12.5	302.072
6	15	366.765

Table 3: Linearity data for impurity-1

S. No.	Concentration (µg/ml)	Peak Area
1	0.25	31.761
2	5	104.375
3	7.5	155.239
4	10	207.427
5	12.5	258.03
6	15	310.493

Table 4: Linearity data for impurity-2

S. No.	Concentration (µg/ml)	Peak Area
1	0.25	23.784
2	5	78.995
3	7.5	117.741
4	10	157.557
5	12.5	196.245
	15	236.321

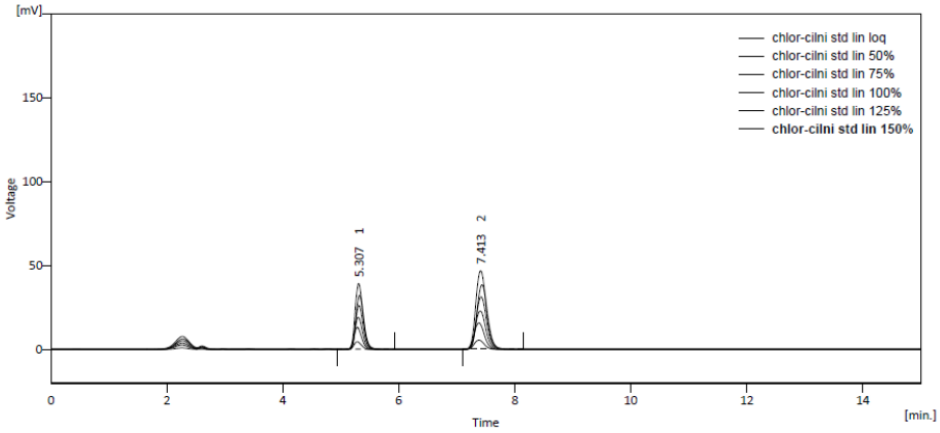

Figure 5: Overlay chromatogramn for mixture of different concentrations for Cilnidipine and Chlorthalidone drugs

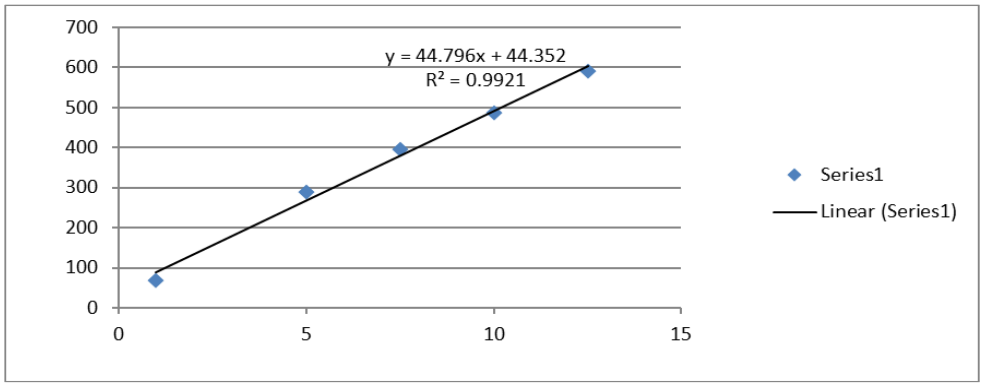

Figure 6: Calibration Curve of Cilnidipine drug (1-15µg/ml)

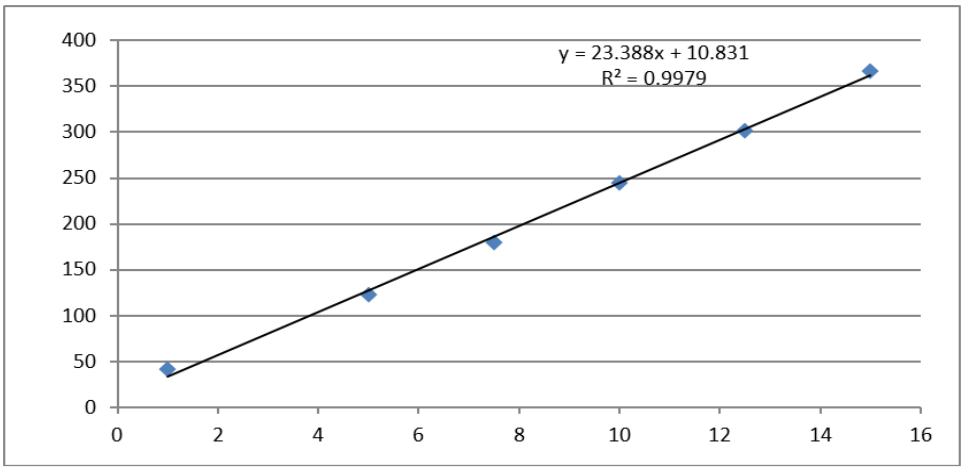

Figure 7: Calibration Curve of Chlorthalidone drug (1-15 µg/ml)

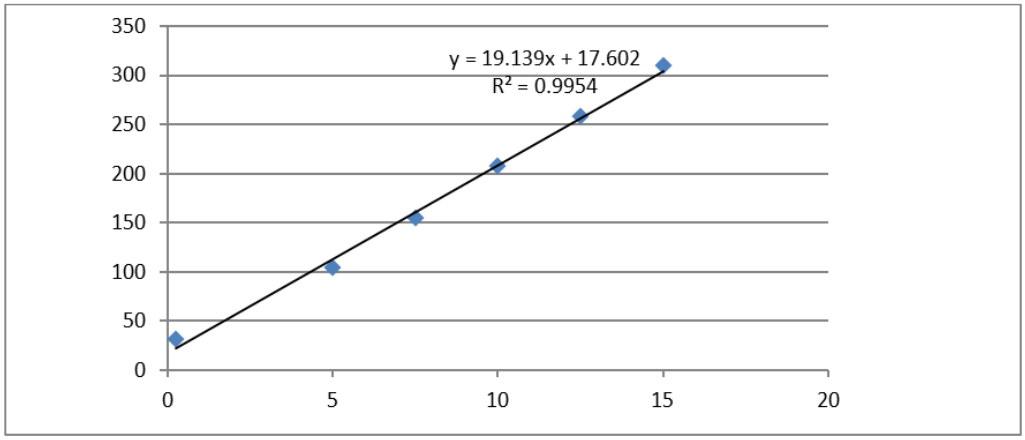

Figure 8: Calibration Curve of impurity-1 (0.25-15µg/ml)

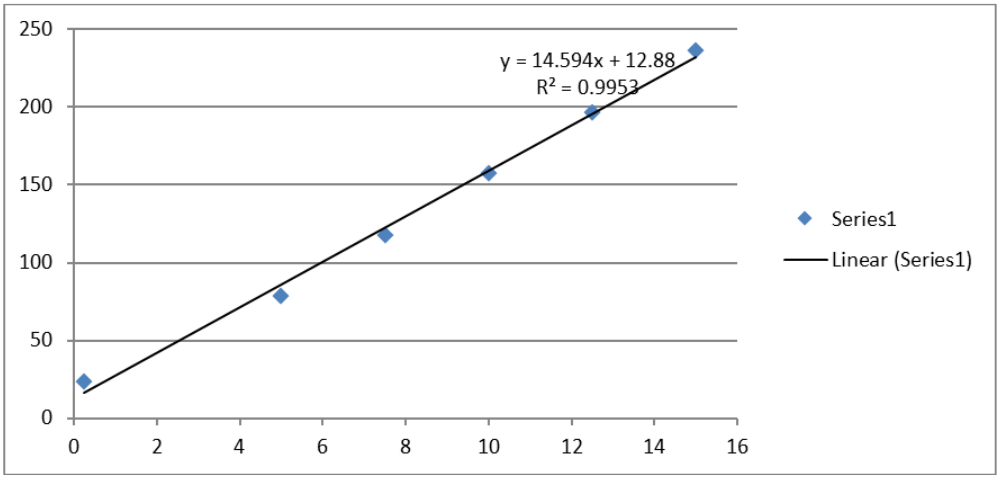

Figure 9: Calibration Curve of impurity-2 (0.25-15µg/ml)

5. Precision

i. Repeatability

Repeatability data of peak area for Cilnidipine, Chlorthalidone drug, impurity-1 and impurity-2, basis on six measurements of same solution of Cilnidipine, Chlorthalidone drug, impurity-1 and impurity-2 are depicted in table. The % RSD for Cilnidipine, Chlorthalidone drug, impurity-1 and impurity-2 components were found to be 1.62, 1.48,1.72 and 1.51 respectively.

Table 5: Repeatability data for Cilnidipine drug

Cilnidipine drug				
S. No.	**Conc (µg/ml)**	**Peak Area**	**Mean ± SD**	**% R.S.D**
1.	10	403.247 390.212 385.905 391.394 393.746 386.457	391.83 ±6.34	1.62

Table 6: Repeatability data for Chlorthalidone drug

Chlorthalidone drug				
S. No.	**Conc (µg/ml)**	**Peak Area**	**Mean ± SD**	**% R.S.D**
1.	10	249.749 241.636 238.993 242.377 243.866 244.362	243.50 ±3.60	1.48

Table 7: Repeatability data for impurity-1

Impurity-1				
S. No.	**Conc (µg/ml)**	**Peak Area**	**Mean ± SD**	**% R.S.D**
1.	10	204.334 203.096 205.773 211.156 208.675 201.793	205.80 ±3.53	1.72

Table 8: Repeatability data for impurity-2

Impurity-2				
S. No.	**Conc (µg/ml)**	**Peak Area**	**Mean ± SD**	**% R.S.D**
1.	10	155.214 154.283 156.286 160.377 158.497 154.914	156.59 ±2.37	1.51

ii. Intraday precision

The results of intraday precision for Cilnidipine and Chlorthalidone drug impurity-1 and impurity-2 components are shown in below table. % R.S.D. for Intraday precision was found to be 1.30-3.35 for Cilnidipinedrug and 1.03-2.72 for Chlorthalidone drug and 1.58-2.88 for impurity-1 and 2.46-4.38 for impurity-2.

Table 9: Intraday precision data for estimation of Cilnidipine drug

Cilnidipine drug			
S. No.	**Concen. (µg/ml)**	**Mean peak area ± SD (n=3)**	**% RSD**
1	0.25	67.92± 2.28	3.35
2	10	395.86± 5.64	1.42
3	15	582.48± 7.60	1.30

Table 10: Intraday precision data for estimation of Chlorthalidone drug

Chlorthalidone drug			
S. No.	**Concen. (µg/ml)**	**Mean peak area ± SD (n=3)**	**% RSD**
1	0	40.34± 1.10	2.72
2	10	245.41± 3.92	1.60
3	15	361.53± 3.83	1.06

Table 11: Intraday precision data for estimation of impurity-1

Impurity-1			
S. No.	**Concen. (µg/ml)**	**Mean peak area ± SD (n=3)**	**% RSD**
1	2.5	31.41± 0.90	2.88
2	10	205.11± 5.96	2.91
3	12.5	307.10 ± 4.86	1.58

Table 12: Intraday precision data for estimation of impurity-2

Impurity-2			
S. No.	**Concen. (µg/ml)**	**Mean peak area ± SD (n=3)**	**% RSD**
1	2.5	23.22± 1.02	4.38
2	10	156.27± 3.85	2.46
3	12.5	232.37± 5.72	2.46

iii. Interday precision

The results for intraday precision for Cilnidipine and Chlorthalidone drug impurity-1 and impurity-2 are shown in table. % R.S.D for interday precision was found to be 0.236-0.978 for Cilnidipine drug and 0.198-0.805 for Chlorthalidone drug and for impurity-1 and for impurity-2 0.384-1.029 for impurity-2.

Table 13: Interday precision results for estimation of Cilnidipine drug

Cilnidipine drug			
S. No.	**Concen. (µg/ml)**	**Mean peak area ± SD (n=3)**	**% RSD**
1	0.25	68.34± 0.94	1.37
2	10	385.01±5.45	1.42
3	15	586.73±6.32	1.08

Table 14: Interday precision results for estimation of Chlorthalidone drug

Chlorthalidone drug			
S. No.	**Concen. (µg/ml)**	**Mean peak area ± SD (n=3)**	**% RSD**
1	0	41.22± 1.04	2.52
2	10	241.33± 1.95	0.81
3	15	365.42 ± 6.98	1.91

Table 15: Interday precision results for estimation of Impurity-1

Impurity-1			
S. No.	**Concen. (µg/ml)**	**Mean peak area ± SD (n=3)**	**% RSD**
1	2.5	31.57 ± 0.77	2.44
2	10	205.57± 3.20	1.56
3	12.5	308.33± 4.18	1.36

Table 16: Interday precision results for estimation of Impurity-2

Impurity-2			
S. No.	**Concen. (µg/ml)**	**Mean peak area ± SD (n=3)**	**% RSD**
1	2.5	22.87± 0.35	1.51
2	10	155.15± 1.20	0.77
3	12.5	232.88± 1.42	0.61

6. Accuracy

The recovery study was carried out at three level of 80%, 100% and 120% of target drug concentration. Percentage recovery for impurity-1 and impurity-2 was found to be

Table 17: Recovery data for impurity-1

S. NO.	**Recovery Level**	**Sample amount (µg/ml)**	**Drug Added (µg/ml)**	**Drug recovered (µg/ml)**	**% Recovery**	**% Average Recovery ± SD**
1		10	8	8.032	100.40	
2	80 %	10	8	7.853	98.16	99.99± 1.66
3		10	8	8.112	101.40	
4		10	10	9.898	98.98	
5	100 %	10	10	10.269	102.69	100.95± 1.85
6		10	10	10.118	101.18	
7		10	12	11.923	99.36	
8	120 %	10	12	12.228	101.90	101.36± 1.77
9		10	12	12.338	102.82	

Table 18: Recovery data for impurity-2

S. NO.	**Recovery Level**	**Sample Amount**	**Amount Added**	**Amount recovered (µg/ml)**	**% Recovery**	**% Average Recovery ±SD**
1		10	8	8.123	101.54	
2		10	8	8.306	103.82	
3	80 %	10	8	7.834	97.93	101.01 ± 2.94
4		10	10	10.294	102.94	
5	100 %	10	10	9.875	98.75	99.78 ± 2.80
6		10	10	9.763	97.63	
7		10	12	12.201	101.68	
8	120 %	10	12	11.877	98.98	100.25 ± 1.35
9		10	12	12.012	100.10	

7. LOD and LOQ

Calibration curve is repeated for five times and the standard deviation (SD) of intercepts was computed. Then the LOD and LOQ value were calculated as follows: LOD = 3.3 * SD/slope value of calibration curve.

LOQ = 10 * SD/slope value of calibration curve.

Where, SD = Standard deviation (SD) of intercepts.

Table 19: Limit of Detection results for Cilnidipine drug and Chlorthalidone impurity-1 and impurity-2

Cilnidipine drug	Chlorthalidone drug	Impurity-1	Impurity-2
LOD = 3.3 * (SD / Slope) = 3.3 * (4.553/39.350) 0.382 µg/ml	LOD = 3.3 * (SD / Slope) = 3.3 * (2.782/24.415) = 0.376µg/ml	LOD = 3.3 * (SD / Slope) = 3.3 * (0.514/20.601) = 0.082µg/ml	LOD = 3.3 * (SD / Slope) = 3.3 * (0.406/15.726) = 0.085µg/ml

Table 20: Limit of Quantitation results for Cilnidipine drug and Chlorthalidone impurity-1 and impurity-2

Cilnidipine drug	Chlorthalidone drug	Impurity-1	Impurity-2
LOQ = 10 * (SD / Slope) = 10 * (4.553/39.350) = 1.157µg/ml	LOQ = 10 * (SD / Slope) = 10 * (2.782/24.415) = 1.139 µg/ml	LOQ = 10 * (SD / Slope) = 10 * (0.514/20.601) = 0.249 µg/ml	LOQ = 10 * (SD / Slope) = 10 * (0.406/15.726) = 0.258µg/ml

8. Robustness

The effect of chromatographic parameters modifications was found to be within the limit as mentioned in the below table. The value of % RSD were found to be less than 2.0%.

Table 21: Robustness data for Cilnidipine drug

S. No.	Flow rate (-0.2 ml/minute) Area	Flow rate (0.2ml/minute) Area	pH (-0.2) Area	pH (+0.2) Area	Mobile phase (- 2) Area	Mobile phase (+2) Area
1	412.13	378.03	409.47	375.62	408.23	374.49
2	415.04	379.55	410.31	369.72	403.26	375.99
3	408.81	370.82	397.48	371.21	410.79	369.54
% R.S.D	0.76	1.24	1.77	0.82	0.94	0.90

Table 22: Robustness data for Chlorthalidone drug

S. No.	Flow rate (-0.2 ml/minute) Area	Flow rate (0.2ml/minute) Area	pH (-0.2) Area	pH (+0.2) Area	Mobile phase (- 2) Area	Mobile phase (+2) Area
1	255.20	234.10	253.56	232.64	252.80	231.95
2	257.00	235.04	252.00	230.69	249.06	232.88
3	251.07	232.61	255.20	229.60	254.58	229.19
% R.S.D	1.20	0.52	0.63	0.67	1.12	0.83

Table 23: Robustness results for impurity-1

S. No.	Flow rate (-0.2 ml/minute) Area	Flow rate (0.2ml/minute) Area	pH (-0.2) Area	pH (+0.2) Area	Mobile phase (- 2) Area	Mobile phase (+2) Area
1	214.40	195.89	217.37	196.68	232.92	206.07
2	210.74	192.57	223.74	204.91	248.49	212.99
3	218.73	191.11	218.03	208.14	237.98	220.09
% R.S.D	1.86	1.27	1.59	2.91	3.31	3.29

Table 24: Robustness results for impurity-2

S. No.	Flow rate (-0.2 ml/minute) Area	Flow rate (0.2ml/minute) Area	pH (-0.2) Area	pH (+0.2) Area	Mobile phase (- 2) Area	Mobile phase (+2) Area
1	162.77	148.88	165.03	149.48	176.89	156.65
2	161.24	143.96	173.66	156.82	186.04	161.93
3	166.05	145.86	172.19	159.50	180.07	165.64
% R.S.D	1.50	1.70	2.71	3.34	2.57	2.80

Table 25: Analysis of available formulation (marketed) by developed and validated chromatography method

Impurity Estimation	
Impurity 1	0.32
Impurity 2	0.67
Unkown single maximum impurity	0.67
Total unknown impurities	1.04

9. Conclusion

The result of method validation proves that the proposed new methods for quantification of impurities in selected drugs are simple, sensitive, accurate, precise and robust in nature. The new developed methods are superior economical methods which can be conveniently adapted in quality control laboratories.

Acknowledgement

The authors are also thankful to ***Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Bhupal Nobles' University, Udaipur,*** Rajasthan for providing necessary equipment, facility & chemicals to complete research work.

References

[1]. National Center for Biotechnology Information. PubChem Compound Database; CID=2732, https://pubchem.ncbi.nlm.nih.gov/compound/2732.

[2]. Singer, Jeff M, Michael J. O'Hare, Carl R. Rehm and John E. Zarembo. "Chlorthalidone." In Analytical Profiles of Drug Substances, 1985;14:1-36. Academic Press.

[3]. https://doi.org/10.1016/S0099-5428(08)60575-4.

[4]. https://en.wikipedia.org/wiki/Cilnidipine.

[5]. Snehal N. Patel, Madhuri A. Hinge, Varsha M. Bhanushali. Development and validation of an UV spectrophotometric method for simultaneous determination of cilnidipine and chlorthalidone. J Pharm Res 2015;9(1):41-45.

[6]. N. Sunitha, Subash C. Marihal, J. Sai sravanthi, A. Venu, BV. Narasimha Rao and B. Appa Rao. Method Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Olmesartan and Cilnidipine in Bulk and Formulations. Int J Pharm Res Allied Sci 2015;4(3):127-135.

[7]. ARUNA G, BHARATHI K, KVSRG PRASAD. Development and Validation of Bioanalytical HPLC Method for Simultaneous Estimation of Cilnidipine and Nebivolol in Human Plasma. Int J Pharm Pharm Sci 2017;9(10):253-259.

[8]. Mayank Bapna, Bhoi Khushbu Girishbhai. Development and Validation of Analytical Method for Simultaneous Estimation of Cilnidipine and Chlorthalidone in their Combined Dosage Form. Int J Ayu Pharm Chem 2015;2(3):51-58.

[9]. International Conference on Harmonisation, Guidelines for the Photostability Testing of New Drug Substances and Products,

[10]. International Conference on Harmonisation, Validation of Analytical Procedures: Text and Methodology, in ICH
[11]. Harmonized Tripartite Guidelines Q2 (R1) 2005.
[12]. 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.
[13]. Rang and Del's Pharmacology; 6th Edn; Churchil levingstone Publishers (P) Ltd, New York, 2004; 277-285.
[14]. Dong WM. Modern HPLC for Practicing Scientists; A Wiley- Inter science publication, USA, 2006; 1-9.
[15]. Kazakevich Y and LoBrutto R. HPLC for pharmaceutical Scientists; A John Wiley and sons, 2007; 1-6.
[16]. Salvador Salado C a, Luz E. Vera-Avila, "On-line solid-phase extraction and high-performance liquid chromatographic determination of chlorthalidone in urine" Journal of Chromatography B, 1997, 690, 195-202
[17]. Madhu BK, Bikshal BK, "Reverse phase-hplc method development and validation for the simultaneous estimation of azilsartan medoxomil and chlortalidone in pharmaceutical dosage forms", Journal of Atoms and Molecules,2012,2(1),117–126
[18]. Chaudhari PP,Bhalerao AV,"Method Validation For Spectrophotometric Estimation Of Cilnidipine", Inernational journal of Pharmacy and Pharmaceutical sciences,2012, 4(5), 96-98. Pawar P, Gandhi SV, Deshpande PB,Vanjar S,Shelar SU, "Simultaneous RP-HPLC estimation of Cilnidipine and telmisartan in combined tablet dosage form", Pelagia Research Library, Der Chemica Sinica, 2013,4(2),6–10.
[19]. Singhal M, Amin S,Kukrety A, Chauhan A, Tiwari VK, Singh RM, mthur SC, Saini PK, Singh GN, Development and validation of RP-UPLC Method Using experimental Desigen approach for simultaneous estimation of candesartan cilexetil and chlorthalidone, 2014, Indian Drugs, 51(11)
[20]. Naazneen S, Sridevi A, Stability-Indicating RP-HPLC Method For The Simultaneous Estimation Of Azilsartan Medoxomil And Chlorthalidone In Solid Dosage Forms ,Inernationalt Journal of Pharmacy and Pharmaceuticl Sciences,2014 ,6(6), 236-243
[21]. Marineni B, Reddy TS, Simultaneous Determination of Telmisartan Impurities and Chlorthalidone impurities by UPLC, International Journal of Pharmaceutical Sciences Review and Research, 2014,26(2), 226-230,
[22]. Bikshal BK, Venkateswara RA and Useni RM, "Stability-indicating reversed-phase HPLC method for the separation and estimation of related impurities of Cilnidipine in pharmaceutical formulations", INDIAN DRUGS, 2018,55 (12),41-49
[23]. HE Ling-yun, HU Gao-yun, ZHOU Yan-bin, LIU Jian-hao, ""Determination of Cilnidipine and its related substances by RP-HPLC", West China Journal of Pharmaceutical Sciences, 2004,01. T Hemant Kumar1, Ayesha Begum, D Gowri Sankar, "Determination of Azilsartan Medoximil and Chlorthalidone in tablets exposed to forced degradation by using RP-HPLC", Biomedical Research 2019,30 (5), 775-785
[24]. P. Ravi Sankar, V. Swathi and P. Srinivasa Babu, Development and Validation of Novel UV and RP-HPLC Methods for Determination of Cilnidipine (A New Generation Ca Channel Blocker) in Pharmaceutical Dosage Form, International Journal of Pharmaceutical Sciences and Research, 2019, 10(4), 1886-1894.