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Method Development and Validation Cilnidipine and Chlorthalidone in Bulk and In Its Pharmaceutical Dosage Forms by Using HPLC as Per ICH Guidelines

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ABSTRACT

New method was established for simultaneous estimation of Clinidipine and Chlorthalidone by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Clinidipine and Chlorthalidone by using Zodiac sil RPC18 4.6×250mm 5µm, flow rate was 1.2 ml/min, mobile phase ratio was (70:30 v/v) methanol: Phosphate bufferpH 3 (pH was adjusted with orthophosphoric acid), detection wavelength was 240nm. The retention times were found to be 2.746 mins and 3.608 mins. The % purity of Clinidipine and Chlorthalidone was found to be 101.27% and 99.97% respectively. The system suitability parameters for Clinidipine and Chlorthalidone such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study Clinidipine and Chlorthalidone was found in concentration range of 50µg-250µg and 5µg-50µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.2 and 0.2, % RSD for intermediate precision was 0.2 and 0.1respectively. The precision study was precise, robust, and repeatable.LOD value was 3.17 and 5.68, and LOQ value was 0.0172 and 0.2125 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Clinidipine and Chlorthalidone in API and Pharmaceutical dosage form.

Keywords: Zodiac sil RPC18 column, Clinidipine and Chlorthalidone, RP-HPLC

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1. Introduction

Cilnidipine decreases blood pressure and is used to treat hypertension and its comorbidities. Due to its blocking action at the N-type and L-type calcium channel, cilnidipine dilates both arterioles and venules, reducing the pressure in the capillary bed. Cilnidipine is vasoselective and has a weak direct dromotropic effect, a strong vasodepressor effect, and an arrhythmia-inhibiting effect.

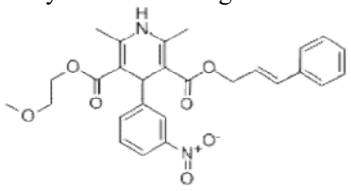

Fig 1: Chemical structure of Cilnidipine

Chlortalidone, also known as chlorthalidone, is a diuretic medication used to treat high blood pressure, swelling including that due to heart failure, liver failure, and nephrotic syndrome, diabetes insipidus, and renal tubular acidosis [1][2]. In high blood pressure it is a preferred initial treatment.

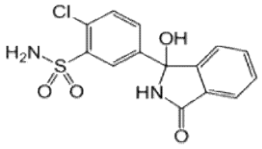

Fig 2: Chemical structure of Chlortalidone

2. Materials and Methods

Instrumentation:

HPLC Auto Sampler : Shimadzu Model number SPD20A, Software LC Solutions, Detector: Photo diode array detector, Thermosil C18 Column (4.0×1.25mm, 5µ), Sonicator: Model number SE60US Enertech , U.V double beam spectrophotometer: PG Instrument Model number T60 Software UV Win5, pH meter: ADWAModel number AD102U, Digital Weighing machine:a Model number ER200A .

Chemicals:

Clinidipine and Chlorthalidone, KH_2PO_4, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid, K_2HPO_4.

Optimized Chromatographic conditions:

Column : Zodiac sil RPC18 4.6×250mm 5µm
Column temperature : Ambient
Wavelength : 240 nm
Mobile phase ratio : 70:30 Methanol: Phosphate buffer
Flow rate : 1.2 ml/min
Auto sampler temperature : Ambient
Injection volume : 10µl
Run time : 10.0 minutes

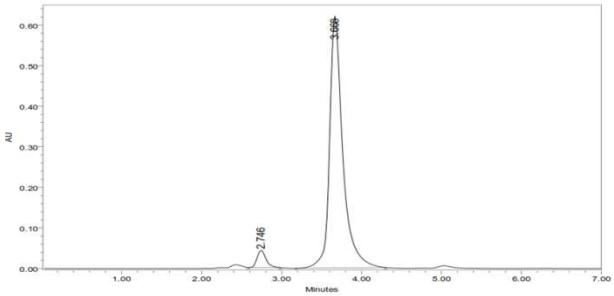

Fig 3: Optimized Chromatogram

Observation:

The retention time of both peaks was good response and height of peaks was good.

Sample solution preparation:

10 mg of Clinidipine and 1 mg Chlorthalidone tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Standard solution preparation

10 mg Clinidipine and 1 mg Chlorthalidone working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Method Validation

- Linearity
- Accuracy
- Precision
- Intermediate Precision
- Limit of Detection
- Limit of Quantification
- Robustness
- System suitability testing

3. Results and Discussion

Table1: Linearity Results for Clinidipine

S.No	Linearity Level	Concentration	Area
1	I	50 ppm	471543
2	II	100 ppm	656277
3	III	150 ppm	794999
4	IV	200 ppm	946124
5	V	250 ppm	1002139
Correlation Coefficient			0.999

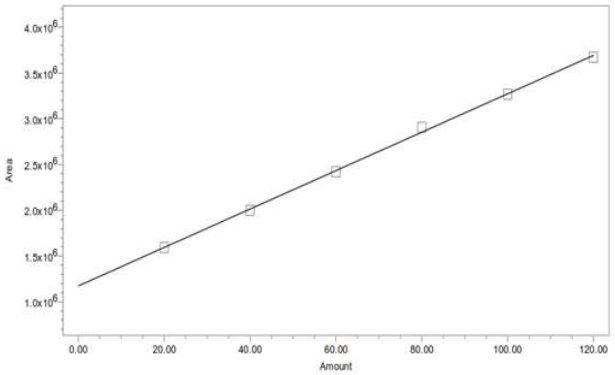

Fig 4: Calibration graph of Clinidipine

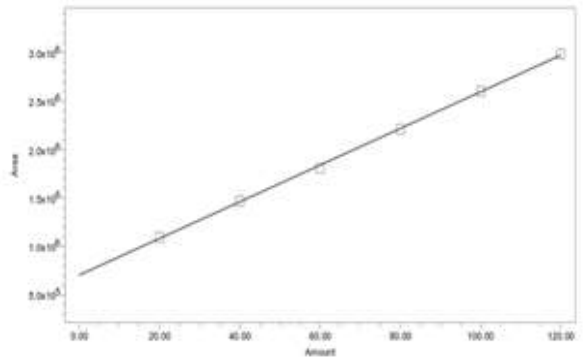

Fig 5: Calibration graph of Chlorthalidone

Table 2: Linearity Results for Chlorthalidone

S.No	Linearity Level	Concentration	Area
1	I	5ppm	56472
2	II	10 ppm	73841
3	III	15ppm	92655
4	IV	20ppm	111541
5	V	25ppm	130567
Correlation Coefficient			0.999

Table 3: Showing accuracy results for Clinidipine

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	656659	5	4.96	99.91%	99.56%
100%	1304258	10	9.98	99.18%	
150%	1854608	15	15.02	99.60%	

Table 4: Showing accuracy results for Chlorthalidone

%Concentration (at specification level)	Average area	Amount added(mg)	Amount found(mg)	% Recovery	Mean recovery
50%	65312	0.5	0.99	99.53%	99.47%
100%	124509	1.0	1.05	99.38%	
150%	178517	1.5	1.495	99.52%	

Table 5: Showing% RSD results for Clinidipine

	Peak name	RT	Area(µV*)	Height(µV)
1	Clinidipine	2.343	1302729	248455
2	Clinidipine	2.344	1302947	248699
3	Clinidipine	2.344	1302947	249526
4	Clinidipine	2.345	1303977	246695
5	Clinidipine	2.345	1303236	250012
Mean			1304529.8	
Std.Dev.			2961.1	
%RSD			0.2	

Table 6: Showing %RSD results for Chlorthalidone

	Peak name	RT	Area(µV*)	Height(µV)
1	Chlorthalidone	3.285	124263	19458
2	Chlorthalidone	3.287	124487	19634
3	Chlorthalidone	3.287	124175	19600
4	Chlorthalidone	3.288	124894	19327
5	Chlorthalidone	3.288	124495	19540

Mean			124462.7	
Std.Dev.			278.6	
%RSD			0.2	

Table 7: Showing results for intermediate precision of Clinidipine

	Peak name	RT	Area(µV*)	Height(µV)
1	Clinidipine	2.342	1305937	247870
2	Clinidipine	2.343	1306476	246764
3	Clinidipine	2.344	1304520	245696
4	Clinidipine	2.344	1300148	247140
5	Clinidipine	2.345	1308271	247280
Mean			1305070.2	
Std.Dev.			3061.8	
%RSD			0.2	

Table 8: Showing results for intermediate precision of Chlorthalidone

	Peak name	RT	Area(µV*)	Height(µV)
1	Chlorthalidone	3.278	122962	19165
2	Chlorthalidone	3.281	122487	19115
3	Chlorthalidone	3.281	122632	19073
4	Chlorthalidone	3.281	122626	19003
5	Chlorthalidone	3.283	122702	19123
Mean			122681.8	
Std.Dev.			174.8	
%RSD			0.1	

Table 9: Showing results for Limit of Detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Clinidipine	382625.50	572175863	3.17
Chlorthalidone	5862.40	467579210	0.0172

Table 10: Showing results for Limit of Quantitation

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Clinidipine	381727.80	583265980	5.80
Chlorthalidone	5681.30	469828490	0.212

Table 11: Showing system suitability results for Clinidipine

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	5339	1.4
2	1	4668	1.3
3	1.2	5216	1.4

Table 12: Showing system suitability results for Chlorthalidone

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	7036	1.3
2	1	6089	1.2
3	1.2	6998	1. 3

Table 13: Showing system suitability results for Clinidipine

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	6232	1.4
2	*Actual	4668	1.3
3	5 % more	6387	1.4

Table 14: Showing system suitability results for Chlorthalidone

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	5437	1.3
2	*Actual	6089	1.2
3	5 % more	4817	1.2

4. Conclusion

All the parameters are validated as per ICH guidelines for the method validation and found to be suitable for routine quantitative analysis in pharmaceutical dosage forms. The result of linearity, accuracy, precision proved to be the result of linearity, accuracy, precision proved to be within limits with lower limits of detection and quantification.

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