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SIMULTANEOUS ESTIMATION OF CILNIDIPINEHYDROCHLORIDEAND CHLORTHALIDONE IN ITS COMBINED DOSAGE FORM BY ABSORBANCE RATIO METHOD

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ABSTRACT

In the quantitative estimation of Cilnidipine and Chlorthalidone in mixture by absorption ratio method, Absorbances was measured at two wavelengths. One is the iso-absorptive point of the components (233 nm) and the other being wavelength of maximum absorbance of cilnidipine hydrochloride (241 nm). From the overlay spectra of the drugs 233nm was selected as iso-absorptive point for absorption ratio method. Different concentrations of cilnidipine hydrochloride and chlorthalidone were prepared in the range of 2 – 10 µg/ml respectively. The absorbance and molar absorptivities were determined at 233 nm and 241 nm for the components and concentrations of cilnidipine and chlorthalidone in combined dosage form were determined. The percentage label claim was found to be 103.16 for cilnidipine hydrochloride and 98 % w/w for chlorthalidone. The validation of the developed method was performed in accordance with ICH guidelines. The accuracy of the proposed method was studied by recovery at three levels. The precision of the proposed method was studied by intraday precision and inter-day precision. The % RSD of the proposed method was found to be < 2%. The linearity was obtained in the concentration range of 2 - 10 µg/ml for cilnidipine hydrochloride and chlorthalidone at 233 nm. The proposed method was found to be accurate and precise, so this method can be used for routine analysis of cilnidipine hydrochloride and chlorthalidone in combined dosage form.

KEYWORDS: Cilnidipine hydrochloride, Chlorthalidone, B.P, ng, nm etc.

INTRODUCTION

Hypertension is defined as either a sustained systolic BP of greater than 140 mm Hgor a sustained diastolic BP of greater than 90 mm Hg. Hypertension is known as a silent killer, Although many of these individuals have no symptoms, chronic hypertension either systolic or diastolic can lead to serious health problems. The incidence of morbidity and mortality significantly decreases when hypertension is diagnosed early and is properly treated. Cilnidipine is a novel dihydro pyridine calcium channel blocker. It is an L- type and N- type calcium channel blocking function. It inhibits cellular calcium influx, thus causing vasodilation. Cilnidipine has greater selectivity for vascular

smooth muscles. It has been included in the list of first line antihypertensive agent by the Chinese guidelines for the prevention and treatment of hypertension. Chlorthalidone is a diuretic drug, that inhibit sodium ion transport across the renal tubular epithelium in the cortical diluting segment of the ascending loop of henle. By increasing the delivery of sodium to the distal renal tubule, chlorthalidone indirectly increases potassium exchange mechanisms. It has a longer duration of action. These combinations improve the tolerability of diuretics by reducing the incidence and magnitude of hypokalemia that is opposed by the aldosterone-inhibiting effect of the RAAS.UV-visible spectrophotometry is one of the most frequently employed techniques in pharmaceutical analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in the solution. A molecule can absorb UV radiation in discrete packets of photon when the energy of the incident radiation is sufficient to induce electronic transition and its associated vibrational and rotational transitions. Electrons are arranged in distinct energy levels in a molecule and absorption of radiation induces transition of electrons into higher energy levels.

MATERIALS AND METHOD

Reagents and chemicals

- Cilnidipine hydrochloride RS
- Chlorthalidone RS
- Methanol HPLC grade from Merck Specialties (P) Ltd Mumbai.

Cilacar - C tablet (commercially available tablet contains 10 mg cilnidipine and 6.25 mg of chlorthalidone), manufactured by J.B Chemicals & Pharmaceuticals. Ltd.

Instruments

- JASCO V 560 double beam spectrophotometer
- Schimadzu analytical balance
- GT sonic, professional ultrasonic cleaner.

METHODOLOGY

1. Preparation of standard stock solution of Cilnidipine RS and chlorthalidone RS separately in methanol.
2. Study of spectral characteristics of Cilnidipine hydrochloride and Chlorthalidone RS in methanol.
3. Study of overlay spectral characteristics of Cilnidipine hydrochloride RS and Chlorthalidone and selection of wavelength.
4. Preparation of calibration curve of Cilnidipine hydrochloride and Chlorthalidone in methanol.
5. Determination of molar absorptivity of Cilnidipine and Chlorthalidone inselected wavelength.
6. Preparation and analysis of standard mixture solution of Cilnidipine hydrochloride and Chlorthalidone by proposed method.
7. Simultaneous estimation of Cilnidipine hydrochloride and Chlorthalidone incombined tablet dosage form.
8. Validation of the proposed method.

1. Preparation of standard stock solution of Cilnidipine hydrochloride and Chlorthalidone in methanol

Cilnidipine hydrochloride

Accurately weighed 10 mg of Cilnidipine hydrochloride RS was quantitatively transferred to a 10 mL standard flask.

It was the dissolved and the solution was made up to the mark using methanol to obtain a concentration of 1000 µg/mL of cilnidipine hydrochloride (solution A).From the stock solution 1 mL was pipetted out to a 100 mL standard flask and made up the volume with methanol. The solution had a concentration of 10 µg/mL of cilnidipine hydrochloride (solution B).

Chlorthalidone

Accurately weighed 10 mg of Chlorthalidone RS was quantitatively transferred to a 10 mL standard flask. It was the dissolved and the solution was made up to the mark using methanol to obtain a concentration of 1000 µg/mL of Chlorthalidone (solution A).From the stock solution 1 mL was pipetted out to a 100 mL standard flask and made up the volume with methanol. The solution had a concentration of 10 µg/mL of chlorthalidone (solution B).

2. Study of spectral characteristics of Cilnidipine hydrochloride and Chlorthalidone separately in methanol

After stabilizing the instrument initially for 30 minutes and blank correction was done using methanol. Then the 10 µg/mL solution of both Cilnidipine hydrochloride and Chlorthalidone was scanned separately in UV region ranging from 200 nm to 400 nm. The absorption spectra were observed with maximum absorption at 233 nm and 275nm for cilnidipine and chlorthalidone respectively.

Study of overlay spectral characteristics of Cilnidipine hydrochloride and Chlorthalidone. After enabling the initial adjustments and blank correction using methanol, the10 µg/mL solution of cilnidipine hydrochloride and chlorthalidone were scanned separately in 200-400 nm in UV region. The overlay spectrum of cilnidipine hydrochloride RS and chlorthalidone RS is shown in figure. From the overlay spectra, two wavelength were selected, one at 233 nm which was the iso-absorptive point and the other at 241 nm, λ_{max} of Cilnidipine hydrochloride.

3. Calibration curves of Cilnidipine hydrochloride and Chlorthalidone in methanol

Accurately pipetted out 2, 4, 6, 8 mL from stock solution B of both Cilnidipine hydrochloride and Chlorthalidone to different 10 mL standard flask and the volume was made up to the mark using methanol. The absorbance of each solution was measured at 233 nm and 241 nm with methanol as blank. Calibration curve of Cilnidipine hydrochloride and Chlorthalidone were plotted at 233 nm and 241 nm are shown in the figures below.

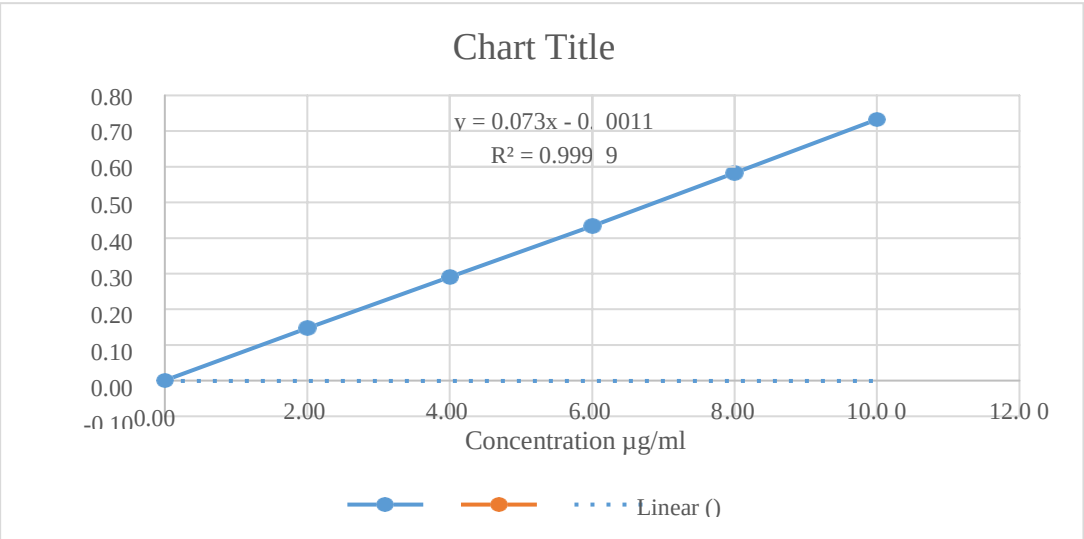

Figure 1: Calibration plot of cilnidipine hydrochloride at 233 nm.

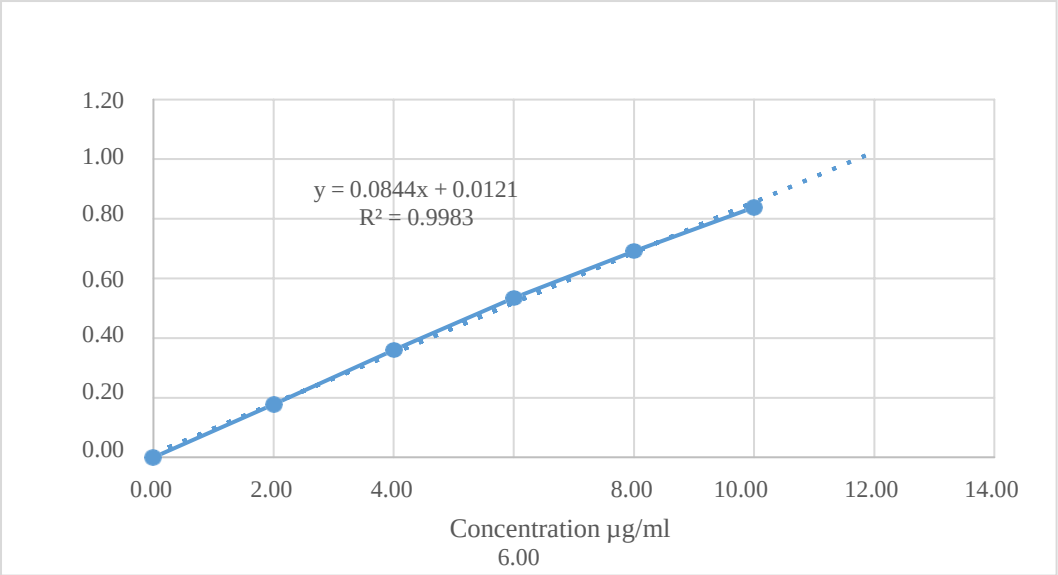

Figure 2: Calibration plot of cilnidipine hydrochloride at 241 nm.

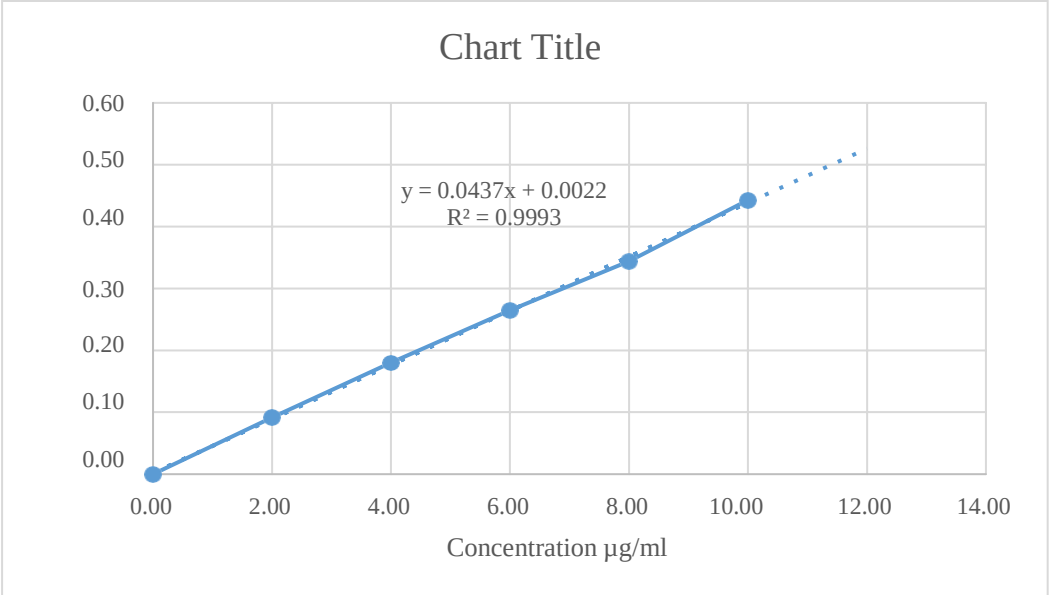

Figure 3: Calibration plot of chlorthalidone 241 nm.

4. Determination of molar absorptivities of Cilnidipine hydrochloride and Chlorthalidone at selected wavelengths

The absorbance of drug solutions, cilnidipine hydrochloride and chlorthalidone in the concentration range 2-10µg/mL was measured at 233 nm and 241 nm. The molar absorptivities for both drugs were calculated using the following equation:

$$A = \varepsilon bc$$

Where,

A = Absorbance of the samplesolution

ε = Molar absorptivity; b=Path length

c = Concentration of the sample

Thus, the molar absorptivity can be determined by the following equation

$$\varepsilon = A/c$$

Results are tabulated in table

Table 1: Cilnidipine hydrochloride absorption data.

Sl. No.	Concentration (µg/ml)	Absorbance	
		233 nm	241 nm
1	2	0.1466	0.1787
2	4	0.2899	0.3607
3	6	0.4328	0.5346
4	8	0.5825	0.6916
5	10	0.7321	0.8390

Table 2: Chlorthalidone absorption data.

Sl. No.	Concentration (µg/ml)	Absorbance	
		233 nm	241 nm
1	2	0.1553	0.0998
2	4	0.3065	0.1798
3	6	0.4534	0.2547
4	8	0.6142	0.3341
5	10	0.7603	0.4429

Table 3: Molar absorptivity data.

Sl. No.	Drug	Molar absorptivity	
		233 nm	241 nm
1	Cilnidipine	0.0723	0.0870
2	Chlorthalidone	0.0765	0.0435

5. Preparation and analysis of standard drug mixture solution of cilnidipine hydrochloride and chlorthalidone in methanol

Weighed accurately 10 mg of cilnidipine and 6.25 mg of chlorthalidone and transferred to a 10mL standard flask. The drug mixture was dissolved insufficient quantity of methanol by sonication for 5 minutes and the volume was made up to the mark with methanol. The solution had a concentration of 1000 µg/mL of cilnidipine and 625 µg/mL of chlorthalidone (solution A). From the above solution, pipetted out 1 mL and transferred to a 100 mL standard flask and made up the volume using methanol. The solution had a concentration of 10 µg/mL of cilnidipine and 6.25 µg/mL of chlorthalidone (solution B).

Six different mixtures containing 10 µg/mL of cilnidipine and 6.25µg/mL of chlorthalidone were prepared similarly and absorbance was measured at two wavelengths, i.e., at iso-absorptive point (233 nm) and λ_{max} of cilnidipine (241 nm). The results are furnished in the table.

Table 4: Absorbance of standard drug mixture of cilnidipine andchlorthalidone.

Sl. No.	Absorbance	
	A1	A2
	233nm (λ1)	241nm (λ2)
1	0.9520	0.7526
2	0.9527	0.7517
3	0.9518	0.7521
4	0.9521	0.7520
5	0.9520	0.7529
6	0.9513	0.7525

Table 5: Assay results of standard drug mixture.

Sl. No.	Amount present in µg/mL		Amount btained in µg/mL		Amount obtained in percentage	
	CILNI	CHLOR	CILNI	CHLOR	CILNI	CHLOR
1	10	6.25	10.3	6.18	103	98
2	10	6.25	10.2	6.15	102	98
3	10	6.25	10.4	6.17	103	98
4	10	6.25	10.3	6.17	103	98
5	10	6.25	10.2	6.15	102	98
6	10	6.25	10.2	6.18	102	98

6. Simultaneous estimation of Cilnidipine hydrochloride and Chlorthalidone incombined tablet dosage form. 7

Table 6: Description of Cilacar – C tablet.

Trade Name	Cilacar – C
Label claim	Cilnidipine hydrochloride 10 mg Chlorthalidone 6.25 mg
Manufactured by	J B Chemicals and pharmaceuticals Ltd.

Contents of ten tablets of CILACAR – C were weighed; average weight of one tablet was calculated and finely powdered with the help of a mortar and pestle. A quantity of powder equivalent to 10 mg of Cilnidipine (containing 6.25 mg of Chlorthalidone) was weighed accurately and transferred to a glass stoppered flask. The powder was extracted initially with methanol by sonication for 10 minutes and filtered through Whatman No.1 filter paper to a 10 mL standard flask. The volume was finally made up to the mark with methanol. The resulting solution had a concentration of 1000 go/mL of Cilnidipine hydrochloride and 625 µg/ml of Chlorthalidone.

From the above solution, accurately pipetted out 1 mL and transferred to a 100 mL standard flask. Then the volume was made up to the mark using methanol toobtain a concentration of 10 µg/mL of Cilnidipine hydrochloride and 6.25 µg/mL of Chlorthalidone.

Six different mixtures were prepared as above and the absorbances of the final solutions were measured at 233 nm and 241 nm. The results are furnished in the table.

Table 7: Absorbance of tablet solution.

Sl. No.	ABSORBANCE	
	233 nm	241 nm
1	0.9517	0.7525
2	0.9513	0.7529
3	0.9520	0.7518
4	0.9528	0.7521
5	0.9520	0.7523
6	0.9514	0.7525

Table 8: Assay results of tablet solution.

Sl. No.	Amount present (Label Claim)mg/tablet		Amount obtainedmg/tablet		Percentage label claim	
	CILNI	CHLOR	CILNI	CHLOR	CILNI	CHLOR
1	10	6.25	10.3	6.16	103	98
2	10	6.25	10.3	6.16	103	98
3	10	6.25	10.3	6.15	103	98
4	10	6.25	10.4	6.18	104	98
5	10	6.25	10.3	6.15	103	98
6	10	6.25	10.3	6.17	103	98

7. RESULTS

Each tablet contains (label claim): Cilnidipine hydrochloride 10 mg.

Chlorthalidone 6.25 mg.

Weight of ten tablet = 0.9920g

Average weight of one tablet = 0.0992g.

Weight equivalent to 10 mg of Cilnidipine hydrochloride= 0.0992 g.

8. Average content per tablet determined by proposed method

Cilnidipine = 0.0103g. Chlorthalidone = 0.00616g.

9. Percentage

Cilnidipine = 103.16 % w/w

Chlorthalidone = 98.56 % w/w

10. Validation of the proposed method

10.1 Accuracy

Accuracy of the proposed method was determined by recovery study. The recovery studies were performed by standard addition method at 80 %, 100%and 120% level and percentage recoveries were calculated. Ten capsules of CILACAR - C (containing 10mg of Cilnidipine hydrochloride and 6.25mg of Chlorthalidone) were weighed; the average weight of tablet was determined and finely powdered using mortar and pestle. Weighed accurately a powder equivalent to 10 mg of cilnidipine hydrochloride (containing 6.25 mg of chlorthalidone) and transferred to aglass stoppered flask. To this added 8mg of cilnidipine and 5 mg of chlorthalidone (80 %). It was then extracted initially with 15 mL of methanol by sonication for 10 minutes. The solution was then transferred to a 100 mL standard flask through Whatmann No.1 filter paper. The residue was further extracted twice with 10 mL methanol and transferred to the standard flask through the same filter paper. The volume was finally made up to the mark with methanol. Accurately pipetted out 1 mL of the above solution to a 100 mL standard flask and the volume were made up to the mark using methanol. He resulting solution had a concentration of 1.8 µg/mL of cilnidipine hydrochloride and 1.125µg/mL of chlorthalidone. The absorbances of the solution were measured at 233 nm and 241 nm in three replicates and the amount recovered was calculated. Similarly, the recovery study carried out for solution of 100 % and 120%. The absorbances were measured in triplicate and the results are furnished in the table. The statistical validation data is shown in table.

Table 9: Data of recovery study.

Sl. No.	Level of % recovery	Absorbance		Drug recovery (%)	
		233 nm	241 nm	CILNI	CHLOR
1	80 %	0.7891	0.7455	103.62	98.61
2		0.7897	0.7462	103.64	98.66
3		0.7894	0.7452	103.66	98.62
1	100 %	0.9514	0.7517	102.88	98.80
2		0.9511	0.7521	102.92	98.82
3		0.9520	0.7512	102.88	98.78
1	120 %	1.0019	0.7701	103.42	98.68
2		1.0014	0.7607	103.38	98.72
3		1.0110	0.7703	103.42	98.68

Table 10: Recovery study-statistical validation data.

Level of % recovery	Mean % Recovery		Standard deviation		% RSD		Coefficient of Variation	
	CILNI	CHLOR	CILNI	CHLOR	CILNI	CHLOR	CILNI	CHLOR
80 %	103.64	98.63	0.0200	0.0264	0.0200	0.0264	0.0002	0.0002
100 %	102.89	98.80	0.0230	0.0240	0.0230	0.0240	0.0002	0.0001
120 %	103.42	98.69	0.0230	0.0230	0.0230	0.0230	0.0002	0.0002

10.2. Intermediate

The intermediate precision was studied by using six determinations of the mixture of 10 µg/mL of Cilnidipine and 6.25 µg/mL of Chlorthalidone. The stock solution wasprepared and analyzed at the same time on three consecutive days. The absorbances of the resulting solution was measured at 233 nm and 241 nm. The variations of the results on three days were analyzed and the statistical validation was done. The results for Day1, Day 2 and Day 3 are furnished in the table 49, 50 and 51 respectively. Thestatistical validation data is furnished in table (61).

Table 11: Intermediate precision – Absorbance.

Sl. No.	Absorbance					
	Day 1		Day 2		Day 3	
	233 nm	241 nm	233 nm	241nm	233nm	241 nm
1	0.9520	0.7523	0.9525	0.7525	0.9518	0.7519
2	0.9527	0.7521	0.9516	0.7519	0.9521	0.7522
3	0.9518	0.7522	0.9520	0.7522	0.9520	0.7526
4	0.9520	0.7529	0.9515	0.7524	0.9520	0.7521
5	0.9514	0.7525	0.9528	0.7518	0.9516	0.7523
6	0.9518	0.7519	0.9514	0.7522	0.9513	0.7521

Table 12: Intermediate Precision-Result of Day 1.

Sl. No.	Amount present (µg/mL)		Amount obtained (µg/mL)		Amount obtained (%)	
	CILNI	CHLOR	CILNI	CHLOR	CILNI	CHLOR
1	10	6.25	10.3	6.17	103.00	98.90
2	10	6.25	10.4	6.17	104.00	98.00
3	10	6.25	10.3	6.17	103.30	98.80
4	10	6.25	10.3	6.18	103.30	98.70
5	10	6.25	10.2	6.18	102.00	98.90
6	10	6.25	10.3	6.15	103.31	98.00

Table 13: Intermediate precision –Result of Day 2.

Sl. No.	Amount present (µg/mL)		Amount obtained (µg/mL)		Amount obtained (%)	
	CILNI	CHLOR	CILNI	CHLOR	CILNI	CHLOR
1	10	6.25	10.3	6.18	103.90	98.90
2	10	6.25	10.2	6.15	102.00	98.00
3	10	6.25	10.3	6.17	103.25	98.80
4	10	6.25	10.2	6.18	102.00	98.90
5	10	6.25	10.4	6.15	104.50	98.70
6	10	6.25	10.2	6.17	102.50	98.00

Table 14: Intermediate precision – Result of Day 3.

Sl. No.	Amount present (µg/mL)		Amount obtained (µg/mL)		Amount obtained (%)	
	CILNI	CHLOR	CILNI	CHLOR	CILNI	CHLOR
1	10	6.25	10.3	6.15	103.50	98.90
2	10	6.25	10.3	6.17	103.50	98.00
3	10	6.25	10.3	6.18	103.25	98.80
4	10	6.25	10.3	6.17	103.00	98.70
5	10	6.25	10.2	6.17	100.00	98.90
6	10	6.25	10.2	6.17	100.50	98.00

Table 15: Statistical validation – Intermediate precision study.

Components	Mean of % label claim	Standard deviation (SD)	Relative standard deviation (% RSD)	Coefficient of variation (CV)
Cilnidipine	103.8833	0.1169	0.1169	0.0012
Chlorthalidone	98.1250	0.5863	0.5863	0.0058

Linearity

The absorbance ratio method showed good linearity for Cilnidipine hydrochloride and Chlorthalidone in the range of 2-10 µg/mL for both. The linearity plot for Cilnidipine hydrochloride and Chlorthalidone are given in the figure 31 & 32. The data showing the linearity of the developed method is shown in the table (53).

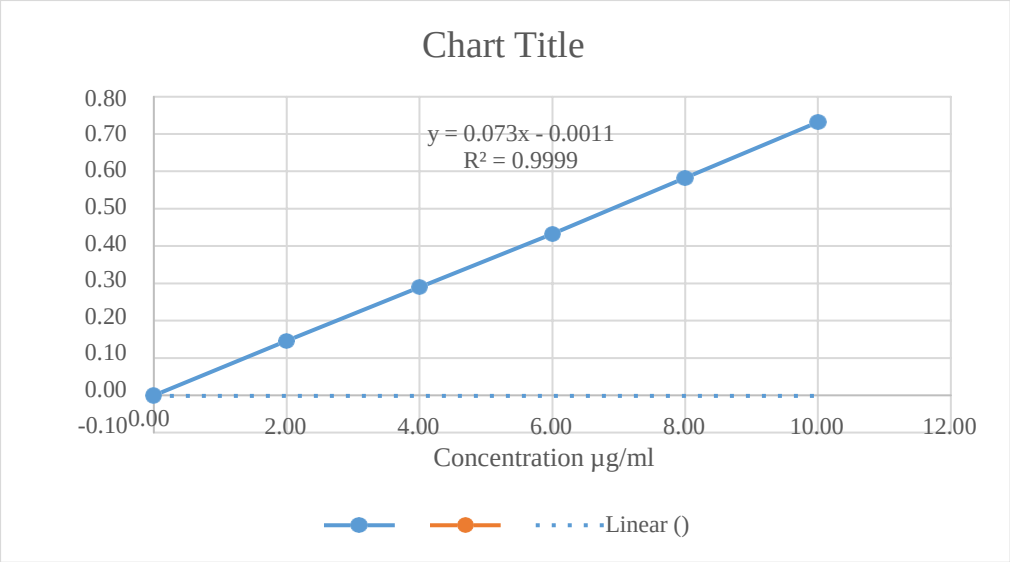

Figure 4: Calibration plot of Cilnidipine hydrochloride at 233 nm.

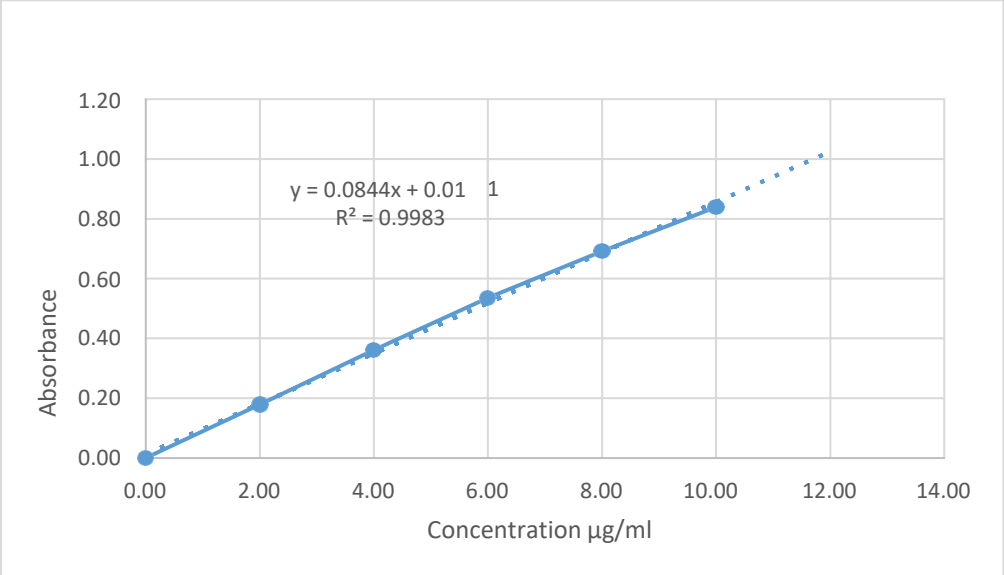

Figure 5: Calibration plot of Chlorthalidone at 241 nm.

Table 16: Linearity data.

Method parameter	Cilnidipine hydrochloride		Chlorthalidone	
	233 nm	241nm	233 nm	241 nm
Linearity (µg/mL)	2-10	2-10	2-10	2-10
Slope	0.0730	0.0844	0.0761	0.0437
Intercept	0.0011	0.0121	0.0013	0.0022
R^2	0.9999	0.9983	0.9990	0.9993

Range

The range of the analytical procedure is normally derived from linearity studies and depends on the intended application of the procedure. From the linearitystudies, it is revealed that the range for the proposed analytical method is as follows.

Table 17: Linearity range of Cilnidipine hydrochloride and Chlorthalidone.

Cilnidipine hydrochloride	2-10 µg/mL
Chlorthalidone	2-120µg/mL

Five calibration curves were dawn at 233 nm and 241 nm for Cilnidipine hydrochloride and Chlorthalidone over the linear range of 2-10 µg/mL for both drugs. From each calibration curve y-intercept and slope were substituted in the given equation for finding LOD and LOQ.

$$LOD = 3.3 \times \frac{\sigma}{S}$$

$$LOQ = 10 \times \frac{\sigma}{S}$$

Where,

σ= Standard deviation of y-intercepts of regression linesS= Slope of the calibration curve

Table 18: LOD and LOQ data.

Drug	Wavelength	Σ	S
Cilnidipine hydrochloride	233 nm	0.0012	0.0730
	241 nm	0.0065	0.0844
Chlorthalidone	233 nm	0.0006	0.0761
	241 nm	0.0065	0.0437

Table 19: LOD and LOQ results.

Method parameters	Cilnidipine		Chlorthalidone	
	233 nm	241 nm	233 nm	241 nm
LOD (µg/mL)	0.0542	0.2541	0.02601	0.4908
LOQ (µg/mL)	0.1643	0.7701	0.0788	1.4871

RESULT AND DISCUSSION

In the quantitative assay of Cilnidipine hydrochloride and chlorthalidone in the mixture by absorption ratio method, absorbance was measured at two wavelengths. One is the iso absorptive point of the components (233 nm). And the other being wavelength of maximum absorbance of cilnidipine hydrochloride (241 nm).From the overlay spectra of the drugs 233nm was selected as isoabsorptive point for absorption ratio method. Different concentrations of cilnidipine hydrochloride and chlorthalidone wereprepared in the range of 2 – 10 µg/ml respectively. The absorbance and molar absorptivities were determined at 233 nm and 241 nm for the components and concentrations of cilnidipine and

chlorthalidone in combined dosage form were determined. The percentage label claim was found to be 103.16 for cilnidipine hydrochloride and 98 % w/w for chlorthalidone. The validation of the developed method was performed in accordance with ICH guidelines. The accuracy of the proposed method was studied by recovery at three levels. The precision of the proposed method was studied by intraday precision and inter-day precision. The % RSD of the proposed method was found to be < 2%. The linearity was obtained in the concentration range of 2 - 10 µg/ml for cilnidipine hydrochloride and chlorthalidone at 233 nm.

SUMMARY AND CONCLUSION

The U.V.spectro-photometric method demonstrated herein are applicable for the simultaneous estimation of Cilnidipine hydrochloride and chlorthalidone in combined tablet dosage form without prior separation. Developed methods were validated according to ICH guidelines. The results obtained from these methods including recovery study were comparable which prove the suitability of the methods for the routine analysis. In order to ensure the integrity of the methods, all procedures were conducted in calibrated equipment and used good quality reagents. The capabilities of the methods are complimentary to each other and were found to be accurate, reproducible, reliable, simple and rapid. The newly developed methods used Microsoft Excel, for manipulation of spectral data, thus eliminating the need for using specific expensive software. The common excipients and other additives usually present in the tablet didn't interfere in the analysis of cilnidipine hydrochloride and chlorthalidone in thesemethods; hence it can be conveniently adopted for the routine quality control analysis of the drugs in combined pharmaceutical formulations. The proposed method was found to be accurate and precise, so this method can be used for routine analysis of cilnidipine hydrochloride and chlorthalidone in combined dosage form.

REFERENCES

1. Nishant T, Arun K, Sathish K D. Development and validation of analytical methods for pharmaceuticals. *J of Analy and Bioanaly Tech*, 2011; 2(5): 1-4.
2. Ashish C, Mittu B, Priyanka C. Analytical method development and validation: A concise review. *J of Analy and Bioanaly Tech.*, 2015: 233-238.
3. Ravisankar P, Navya C N, Pravallika D. A review on step-by-step analytical method validation. *JOSR J of Pharma.*, 2015; 5(10): 7-19.
4. Analytical procedures and method validation for drugs and biologics: Guidance for industry. U S Department of Health and Human Services, 2015: 2-8.
5. Siddiqui M R, Rahman N. Analytical techniques in pharmaceutical analysis: A review. *Arabian J of Che.*, 2017; 10(1): S1409-SI42.
6. Sharma B K, Instrumental methods of chemical analysis. GOEL publishing house, 2015; 13: p.286-385.
7. Dr. Ravisankar S. Text Book of Pharmaceutical Analysis, 2012; 4: p.l81-18.15.
8. Mehta A. Ultraviolet- Visible spectroscopy- Principle. Analytical chemistry, 2011.
9. Chaudhary J, Jain A, Saini V. Simultaneous estimation of multicomponent formulation by UV-visible spectroscopy: An overview. *Inter Res. Journal of Pharma*, 2011; 2(12): 81-83.
10. Guidance for industry: Bio analytical method validation. U S Department ofHealth and Human Services, 2013; 1: 4-15.
11. Validation of analytical procedures: Text and methodology Q2Rl. ICH Harmonized Tripmtite Guideline, 205; 4: 1-13.

12. Guidance for industry.Q2B validation of analytical methodology. ICH.l996:1-8. Snehal N Patel, Madhuri A. Development and Validation of an UV spectrophotometric method for simultaneous determination of cilnidipine and chlorthalidone. *Journal of pharmacy research*, 2015, 9(1): 41-45.