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Development and Validation of UV Spectrophotometric method for the simultaneous estimation of Cilnidipine and Telmisartan in bulk and tablet dosage form utilising Simultaneous Equation and Absorbance Ratio Method.

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ABSTRACT:
The present work involves development and validation of simple, precise and accurate UV - spectrophotometric method (simultaneous equation and absorbance ratio method) for simultaneous estimation of Cilnidipine and Telmisartan in bulk and their combined tablet dosage form.

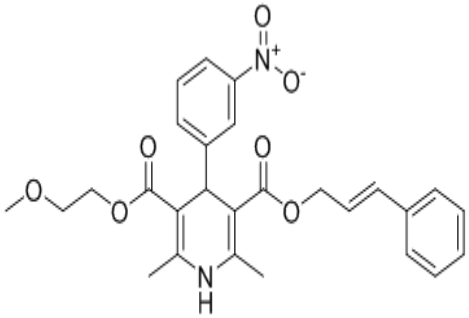
Method: UV- spectrophotometric method,
Simultaneous equation method, estimation of Cilnidipine and Telmisartan was carried out at 241nm and 296.6 nm respectively using methanol: distilled water (80:20) as a solvent. The percentage purity was found to be 99.33 - 99.63% for CIL and 99.50 - 99.75% for TEL.
Absorbance ratio method, estimation of Cilnidipine and Telmisartan was carried out at 241nm and 296.6 nm respectively using methanol: distilled water (80:20) as a solvent. The 270 nm is Q- point (λ_1) and λ_{max} of Telmisartan 296.6 nm is used as λ_2. The percentage purity was found to be 98.48 - 99.81 % for CIL and 99.96 - 100.03% for TEL.
The results have been validated statistically as per ICH guidelines.

Keywords: cilnidipine, telmisartan, uv-spectroscopic method, simultaneous equation method, absorbance ratio method, validation, ICH guidelines.

I. INTRODUCTION:

Cilnidipine is used in treatment of hypertension. It is a dual blocker of L – type voltage – gated calcium channels in the vascular smooth muscles and N – type calcium channels in sympathetic nerve terminals that supply blood vessels & dilates efferent and afferent arterioles. Chemically it is described as 2 – methoxyethyl (2E) – 3 – phenyl – 2 – propen – 1 – yl 2, 6 – dimethyl – 4 – (3 – nitrophenyl) – 1, 4 – dihydro – 3, 5 – pyridine dicarboxylate[39-42].

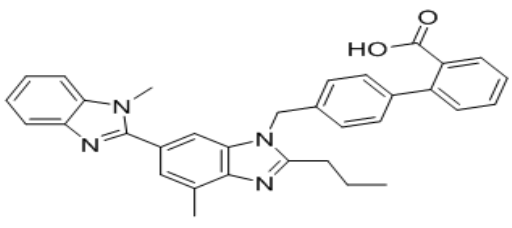
Chemical structure of Cilnidipine



Cilnidipine

Telmisartan is used in treatment of hypertension. It is an angiotensin – II receptor antagonist that shows high affinity for the angiotensin II receptor type 1 (AT1) with a binding affinity 3000 times greater than AT2. Chemically it is known as 4 – ((2 – n – propyl – 4 – methyl – 6 – (1 – methylbenzimidazole – 2 – yl) – benzimidazole – 1 – yl) methyl) diphenyl – 2 – carboxylic acid[41-42].

Chemical structure of Telmisartan



Telmisartan

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II. MATERIAL AND METHODS:

Spectrophotometric measurements were performed on Shimadzu UV - visible double beam spectrophotometer (model - UV - 2600).

Chemicals and reagents:

Cilnidipine bulk drug was obtained from Pure Chem pvt. Ltd., Ankleshwar (Gujarat). The bulk drug Telmisartan was obtained from Aarti Pharma, Mumbai (Maharashtra). The pharmaceutical dosage form used in study was Cilacar - T labeled to contained 10mg Cilnidipine and 40 mg Telmisartan was procured from market (mfg. By J.B. Chemicals and Pharmaceuticals Ltd., Mumbai. The methanol: distilled water (80:20) were used as solvents throughout the experimentation.

Method development:

➢ Preparation of standard stock solution:

Standard stock solution of CIL and TEL were prepared accurately weighing 10 mg of CIL and TEL to 100 mL volumetric flask containing solvent. The drugs were shaked and volumes were made up to mark with solvent to get the concentration of 100µg/mL for each CIL and TEL.

➢ Selection of analytical wavelength:

1mL of the standard stock was pipette out and transfers to 10 mL volumetric flask and volume was made up to mark with methanol: distilled water (80: 20). The solution was scanned in the wavelength range of 200-400 nm and λ_{max} for both CIL and TEL were determined separately. Results were shown in Fig. No.1 & 2.

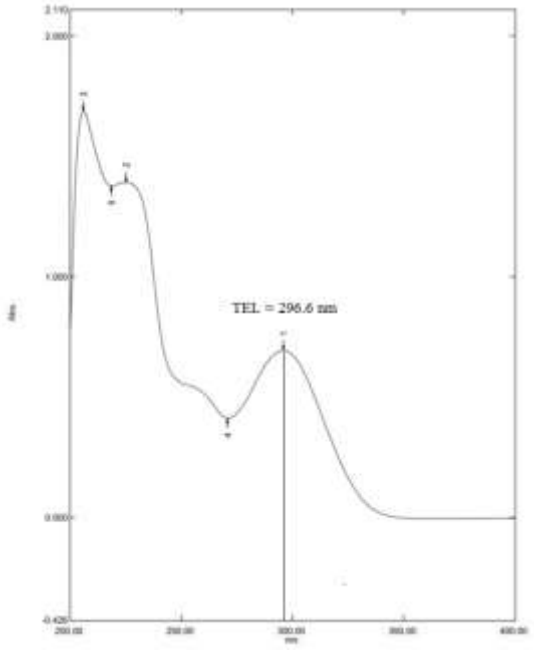

Fig.1 spectra of TEL (5 µg/mL)

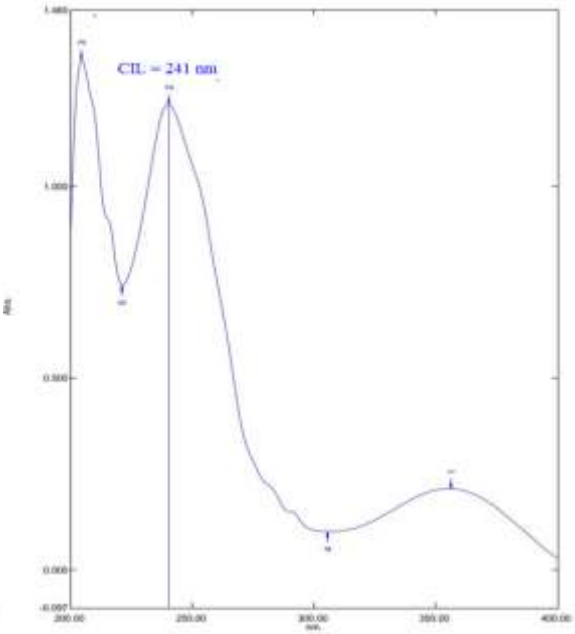

Fig.2 spectra of CIL (5 µg/mL)

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➢ Selection of concentration range and preparation of calibration curve:

Aliquots portion 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 mL were pipette out from the standard stock solution and transferred to series of 10 mL volumetric flask and volume were made with solvent to get the conc. range from 2-14 µg/mL. The absorbance were measured three times for each conc. Absorbance of each solution was measured against methanol: distilled water (80: 20) as blank at 241 nm & 270 nm for CIL for Simultaneous Equation Method and Absorbance Ratio Method resp. Results were shown in Fig. No.3, 4.

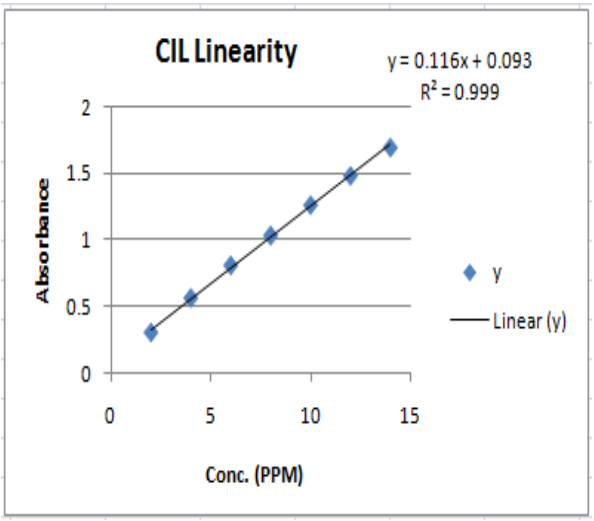

Fig.3 CIL linearity for simultaneous equation

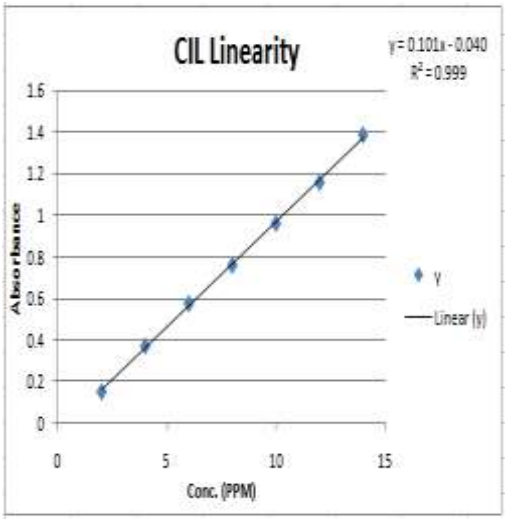

Fig.4 CIL linearity for absorbance ratio

Aliquots portion 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 mL were pipette out from the standard stock solution and transferred to series of 10 mL volumetric flask and volume were made with solvent to get the conc. range from 5-35 µg/mL. The absorbance was measured three times for each. Absorbance of each solution was measured against methanol: distilled water (80:20) as blank at 296.6 nm & 270 nm for TEL for Simultaneous Equation Method and Absorbance Ratio Method resp. Results were shown in Fig. No. 5, 6

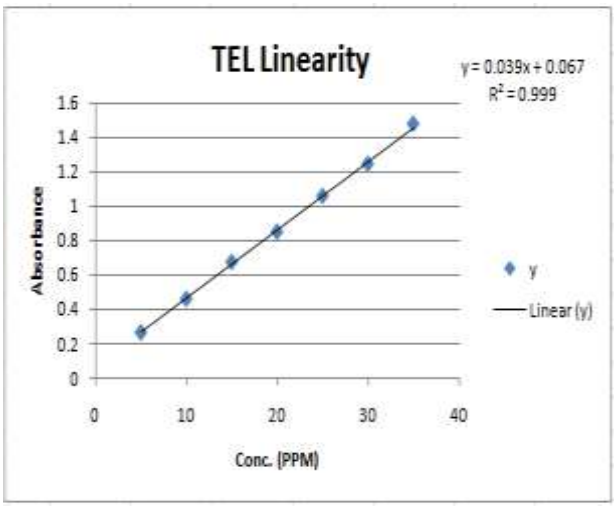

Fig.5 TEL linearity for simultaneous equation

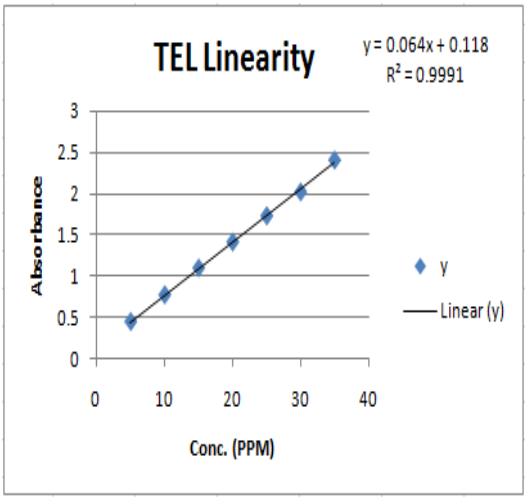

Fig.6 TEL linearity for absorbance ratio

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➢ Analysis of mixed standard of CIL and TEL:

Standard mixtures were made in the linearity range of CIL and TEL i.e. 2-14 µg/mL of CIL and 5-35 µg/mL of TEL form the working stock solution of CIL and TEL (100 µg/mL). Also the standard mixture were prepared from the standard stock solution and by using two sampling wavelength 241 nm (λ_2) for CIL and 296.6 nm (λ_1) for TEL for Simultaneous Equation Method & 270 nm (iso – absorptive point) i.e. λ_1 and 296.6 nm (λ_2) for Absorbance Ratio Method. The conc. of CIL and TEL were calculated by putting absorbance values in the equations given below. Amount of each drug was calculated by using formula

For Simultaneous Equation Method,

$$Cx = \frac{A_2 . ay_1 - A_1 . ay_2}{ax_2 . ay_1 - ax_1 . ay_2} \qquad Cy = \frac{A_1 . ax_2 - A_2 . ax_1}{ax_2 . ay_1 - ax_1 . ay_2}$$

Where,
Cx & Cy are conc. of CIL and TEL resp.
A_1 & A_2 = absorbance of mixture at 296.6 nm and 241 nm resp.
ax_1 & ax_2 = absorptivity of CIL at 296.6 nm and 241 nm resp.
ay_1 & ay_2 = absorptivity of TEL at 296.6 nm and 241 nm resp.

For Absorbance Ratio Method,

$$Cx = \frac{Qm - Qy}{Qx - Qy} \times \frac{A_1}{ax_1} \qquad Cy = \frac{Qm - Qx}{Qy - Qx} \times \frac{A_1}{ay_1}$$

Where,
Cx & Cy are conc. of CIL and TEL resp.
Qm = ratio of absorbances at 296.6 nm and 270 nm resp.
Qx = ratio of absorptivity of CIL at 296.6 nm and 270 nm resp.
Qy = ratio of absorptivity of TEL at 296.6 nm and 270 nm resp.
A_1 = absorbance of mixture at 270 nm.
ax_1 = absorptivity of CIL at 270 nm.
ay_1 = absorptivity of TEL at 270 nm.

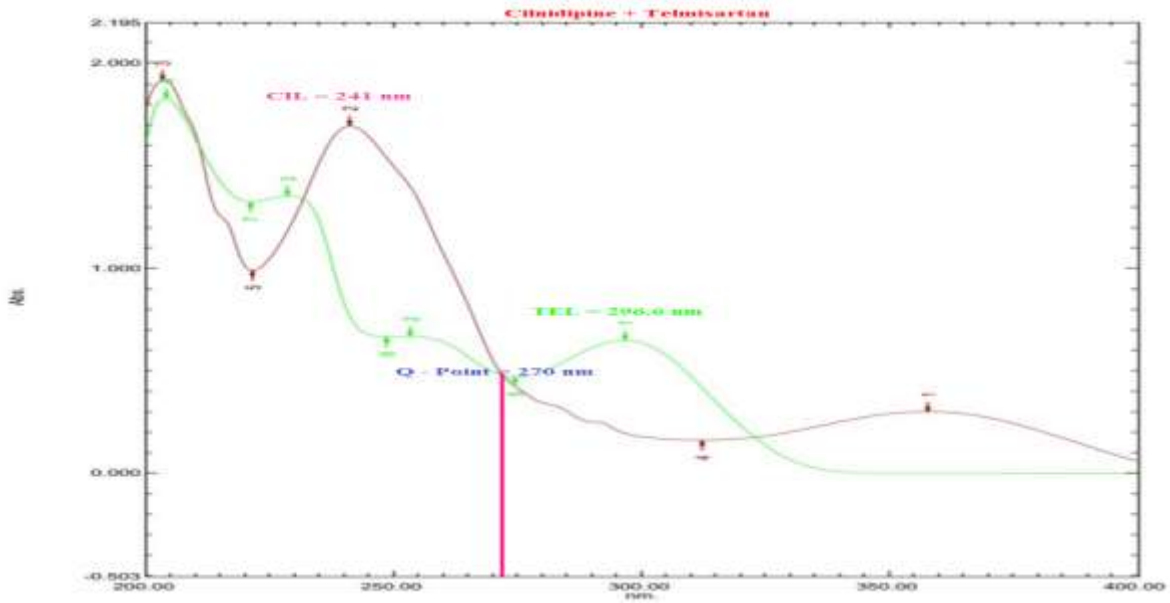

Fig.7 Overlain Spectra of CIL (10 µg/mL) and TEL (10 µg/mL)

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➢ Recovery studies of mixed standards:

Recovery studies were carried out by addition of standard drug solution to the mixed standard solutions at three different levels 80 %, 100 % and 120 %. In 100 % recovery study of CIL the standard drug solution added was 3 µg/mL and in 80 % and 120 % recovery study, 2.4 µg/mL and 3.6 µg/mL was added. In 100 % recovery study of TEL the standard drug solution added was 12 µg/mL and in 80 % and 120 % recovery study, 9.6 µg/mL and 14.4 µg/mL was added. The mixed sample solutions then analyzed to get the spectrum, the absorbance was measured at 296.6 nm (λ_1) and 241nm (λ_2) & 270 nm (λ_1) and 296.6 nm (λ_2) for Simultaneous Equation Method & Absorbance Ratio Method resp. and conc. for each drug in mixture was determined. At each levels of the amount, three determinations were performed.

➢ Analysis of Tablet formulation:

Twenty tablets were weighed and finely powdered. Equivalent to 40 mg of TEL was weighed and transferred to a 100 mL volumetric flask containing 70 mL of methanol:distilled water (80: 20) and sonicated for 15 minutes with intermittent shaking. The solution was filtered through 0.45 µm membrane filter and volume was made up to mark with solvent and mixed to get 100 µg/ml. An aliquot of tablet stock solution 1.2 mL was transferred to 10 mL volumetric flask and volume was made up to mark with methanol-distilled water (80:20) to get conc. of 12 µg/mL of TEL and 3.0 µg/mL of CIL.

Method Validation:

FOR API

According to ICH Q2 (R1) guidelines the developed method was validated to assure the reliability of results of the analysis for different parameters like linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), specificity, ruggedness, and robustness.

1. Linearity

The linearity was accessed by plotting calibration curve for both CIL and TEL. For these, six different conc. of CIL and TEL ranging from 2-14µg/mL and 5-35µg/mL were prepared and analyzed respectively.

S.N.	Parameters	Simultaneous Equation Method		Absorbance Ratio Method	
		CIL	TEL	CIL	TEL
1.	λmax (nm)	241	296.6	270	270
2.	Beer's law limit (PPM)	2-14 (PPM)	5-35 (PPM)	2-14 (PPM)	5-35 (PPM)
3.	Regression equation [y]	y = 0.116x + 0.093	y = 0.064x + 0.118	y = 0.101x + 0.040	y = 0.039x + 0.067
4.	Correlation coefficient (r2)	R^2 = 0.999	R^2 = 0.9991	R^2 = 0.999	R^2 = 0.999
5.	Limit of detection (LOD) (µg/ML)	0.0178	0.0322	0.0375	0.0973
6.	Limit of quantitation (LOQ) (µg/mL)	0.0539	0.0978	0.1138	0.2948

Table No.01

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2. Precision

Intra-day precision (Repeatability) was performed by taking three different conc. (3, 4, 5 µg/mL of CIL and 12, 15, 18 µg/mL of TEL) covering specified range in the triplicates and were analyzed three times within a day with same operator and with same equipment. Inter-day precision was determined by analyzing three different conc. (3, 4, 5 µg/mL of CIL and 12, 15, 18 µg/mL of TEL) in triplicates on three different days within same laboratory conditions. (n=3)

Intra-day							
Cilnidipine				Telmisartan			
Conc. (PPM)	% Conc.	SD	%RSD	Conc. (PPM)	% Conc.	SD	%RSD
3	99.33	0.000601	0.1368	12	99.58	0.000707	0.08008
4	101	0.000441	0.07849	15	99.46	0.000707	0.06588
5	101.20	0.000601	0.08822	18	100.05	0.000707	0.05563
Inter-day							
3	99.33	0.000707	0.16107	12	99.66	0.000667	0.07548
4	101	0.000707	0.12589	15	98.66	0.000441	0.04108
5	101.60	0.00050	0.07342	18	100	0.000782	0.0615

Table No.02

3. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness was performed by change in the temperature of surrounding, changing strength of solvent, change in the λ max, different instrument. (n=3)

Drug	Conc. (PPM)	% Conc.	SD	%RSD	S.E.M.
CIL	3	100	0.00057	0.1307	0.00033
	4	101.25	0.001	0.1776	0.00057
	5	101.60	0.00057	0.0844	0.00033
TEL	12	99.75	0.00057	0.0652	0.00033
	15	100	0.00057	0.0535	0.00033

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	18	102.77	0.00057	0.0453	0.00033

Table No.03

4. Ruggedness

It is the degree of reproducibility of the test results under variety of conditions like different analyst. (n=3)

Analyst – I							
Cilnidipine				Telmisartan			
Conc. (PPM)	%Conc.	SD	%RSD	Conc. (PPM)	%Conc.	SD	%RSD
3	98.33	0.001	0.2314	12	98.58	0.00057	0.0665
4	98.75	0.00057	0.1046	15	98.93	0.001	0.0943
5	99	0.00057	0.0865	18	99.38	0.00057	0.0462
Analyst – II							
3	101.33	0.00057	0.1292	12	100.08	0.001	0.1127
4	101.50	0.001	0.1769	15	99.80	0.00057	0.0536
5	102	0.001	0.1469	18	100.38	0.001	0.0784

Table No.04

5. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, excipients, etc. The specificity of method was determined by checking the interference of excipients with analyte. Talc was chosen as excipient for specificity. Its 100 µg/mL solution used to make the dilutions of specificity. (n=3)

Amount of drug (PPM)	Amount Found	% Found	SD	% R.S.D.	S.E.M.
CIL 3	2.98	99.33	0.00057	0.131	0.00033
	3.04	101.33	0.00057	0.129	0.00033
	3.02	100.66	0.001	0.225	0.00057
TEL 12	11.94	99.50	0.00057	0.065	0.00033
	11.96	99.66	0.00057	0.065	0.00033
	11.98	99.83	0.001	0.113	0.00057

Table No.05

B. FOR TABLET

According to ICH Q2 (R1) guidelines the developed method was validated to assure the reliability of results of the analysis for different parameters like linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), specificity, ruggedness, and robustness.

1. Precision

Intra-day precision (Repeatability) was performed by taking three different conc. (3, 4, 5 µg/mL of CIL and 12, 16, 20 µg/mL of TEL) covering specified range in the triplicates and were

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analyzed three times within a day with same operator and with same equipment. Inter-day precision was determined by analyzing three different conc. (3, 4, 5 µg/mL of CIL and 12, 16, 20 µg/mL of TEL) in triplicates on three different days within same laboratory conditions. (n=3)

Intra-day							
Cilnidipine				Telmisartan			
Conc. (PPM)	% Conc.	SD	%RSD	Conc. (PPM)	% Conc.	SD	%RSD
3	99	0.001414	0.17324	12	100.16	0.001537	0.12935
4	100	0.001364	0.12517	16	99.87	0.001986	0.12563
5	100.60	0.001394	0.10236	20	99.55	0.001509	0.07647
Inter-day							
3	99.33	0.002828	0.34591	12	100.33	0.002833	0.23818
4	100	0.002224	0.20385	16	99.93	0.002224	0.14066
5	100.60	0.002398	0.17588	20	99.60	0.002819	0.14269

Table No.06

2. Accuracy

Accuracy of an analytical procedure is the closeness of test results to the true value. Accuracy was determined by standard addition method. The study was determined by spiking known amount of standard stock (2.4, 3.0, 3.6 µg/mL of CIL and 9.6, 12, 14.4 µg/mL of TEL) to the test solution prepared from tablet formulation at three different spiking level 80 %, 100 % and 120 % of target concentration.

Amount of drug (PPM)	Amount Spiked (PPM)	% Recovered	SD	% R.S.D.
CIL 3	2.4	98.39	0.00057	0.039
	3	98.66	0.00057	0.035
	3.6	98.38	0.00057	0.032
TEL 12	9.6	99.81	0.00057	0.034
	12	99.77	0.00057	0.031
	14.4	99.91	0.001	0.048

Table No.0

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3. Limit of Detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Results were shown in Table No.01. LOD was calculated using the following formula,

LOD = 3.3 σ/S

Where, σ is the standard deviation of the response and S is the slope of the calibration curve.

4. Limit of Quantitation (LOQ)

The limit of quantitation is ability of analytical procedure that the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. Results were shown in Table No.01.

LOQ was calculated using the following formula,

LOQ = 10 σ/S

Where, σ is the standard deviation of the response and S is the slope of the calibration curve.

5. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness was performed by change in the temperature of surrounding, changing strength of solvent, change in the λ max, different instrument. (n=3).

Drug	Conc. (PPM)	%Amount found	SD	%RSD	S.E.M.
CIL	3	98.33	0.00115	0.1419	0.00066
	4	100	0.00115	0.1059	0.00066
	5	100.40	0.001	0.0734	0.00057
TEL	12	100.08	0.00057	0.0486	0.00033
	16	99.81	0.00057	0.0365	0.00033
	20	99.55	0.00153	0.0775	0.00088

Table No.08

6. Ruggedness

It is the degree of reproducibility of the test results under variety of conditions like different analyst. (n=3).

Analyst – I							
Cilnidipine				Telmisartan			
Conc. (PPM)	%Conc.	SD	%RSD	Conc. (PPM)	%Conc.	SD	%RSD
3	98	0.00153	0.1885	12	99.83	0.001	0.0844
4	99.50	0.001	0.0920	16	99.56	0.00115	0.0732
5	100.40	0.00057	0.0425	20	99.10	0.00057	0.0293
Analyst – II							
3	99.33	0.00057	0.0705	12	100.33	0.00115	0.0972

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4	100.25	0.00152	0.1398	16	100.06	0.00057	0.0365
5	101	0.001	0.0733	20	99.65	0.00153	0.0774

Table No.09

7. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, excipients, etc. The specificity of method was determined by checking the interference of excipients with analyte. Talc was chosen as excipient for specificity. Its 100 µg/mL solution used to make the dilutions of specificity. (n=3)

Amount of drug (PPM)	Amount Found	% Found	SD	% R.S.D.	S.E.M.
CIL 4	3.97	99	0.001	0.122	0.00057
	4.01	100.25	0.00115	0.105	0.00066
	4.03	100.60	0.00057	0.042	0.00033
TEL 16	16.02	100.16	0.00153	0.128	0.00088
	15.97	99.81	0.001	0.063	0.00057
	15.91	99.55	0.00057	0.029	0.00033

Table No.10

III. RESULT & DISCUSSION:

In the present research work, a successful attempt was made for determination of Cilnidipine and Telmisartan in bulk and tablet dosage form by UV-Visible Spectrophotometric.

A. Simultaneous equation method by UV

1. A simple, reproducible and specific UV Spectroscopic method was developed for CIL and TEL in bulk drug.
2. The λmax of CIL and TEL were found at 241 nm and 296.6 nm resp. by UV Spectrophotometer.
3. Assay of marketed tablet formulation was done and percentage purity was found to be 99.33-99.63 % for CIL and 99.50-99.75 % for TEL.
4. The developed method was validated for linearity, precision, LOD, LOQ and accuracy, specificity and robustness.
5. Linearity was obtained in the range of 2-14 µg/ml (r2=0.999) for CIL and 5-35µg/ml (r2=0.9991) for TEL.
6. LOD were found to be 0.0178 µg/ml and 0.0322 µg/ml for CIL and TEL resp.
7. LOQ were found to be 0.0539 µg/ml and 0.0978 µg/ml for CIL and TEL resp.
8. % RSD values of Repeatability, intra-day and inter-day precision was found to be less than 2 and it showed that method was precise.
9. Percentage recovery was found to be 98.50-100.0 % for CIL and 99.50-99.95 % for TEL, thus method was accurate.
10. There was not much change in % RSD of CIL and TEL by change in analyst and instrument, thus developed method was found to be robust.

B. Absorbance Ratio method by UV

1. A simple, reproducible and specific UV Spectroscopic method was developed for CIL and TEL in bulk drug.
2. The λmax of CIL and TEL were found at 241 nm and 296.6 nm resp. by UV Spectrophotometer.
3. Assay of marketed tablet formulation was done and percentage purity was found to be 98.33-99.0% for CIL and 99.63-99.83 % for TEL.

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4. The developed method was validated for linearity, precision, LOD, LOQ and accuracy, specificity and robustness.
5. Linearity was obtained in the range of 2-14 µg/ml (r2=0.999) for CIL and 5-35µg/ml (r2=0.999) for TEL.
6. LOD were found to be 0.0375 µg/ml and 0.0973 µg/ml for CIL and TEL resp.
7. LOQ were found to be 0.1138 µg/ml and 0.2948 µg/ml for CIL and TEL resp.
8. % RSD values of Repeatability, intra-day and inter-day precision was found to be less than 2 and it showed that method was precise.
9. Percentage recovery was found to be 98.48-99.81 % for CIL and 99.96-100.03 % for TEL, thus method was accurate.
10. There was not much change in % RSD of CIL and TEL by change in analyst and instrument, thus developed method was found to be robust.

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REFERENCES:

[1]. Recent applications of analytical techniques for quantitative pharmaceutical analysis: a review by Rudy Bonfilio et al., from WSEAS TRANSACTIONS on BIOLOGY and BIOMEDICINE, (2010), 4 (7), 316 – 317, BRAZIL.

[2]. Recent advances of capillary electrophoresis in pharmaceutical analysis by L. Suntornsuk, from Analytical and Bioanalytical Chemistry, (2010), 1 (398), 29 –52.

[3]. Capillary electrophoresis in chemical/pharmaceutical quality control by A. Pluym et al., from Trends in Analytical Chemistry, (1992), 1 (11), 27 – 32.

[4]. Beckett A. H., Stenlake J. B., Practical Pharmaceutical Chemistry, Fourth Edition part II, (2004), CBS Publishers and Distributors, 284-300, 162-163.

[5]. Chatwal G. R., Anand S. K., Instrumental Methods of Chemical Analysis, Fifth Edition, 2008, Himalaya Publishing House, 2.108-2.124.

[6]. Kenkel J., Analytical Chemistry For Technicians, Third Edition, 2009, Published by CRC Publication, 2 – 4.

[7]. Kasture A. V., Mahadik K. R., Wadodkar, S. G., More, H. N., Pharmaceutical Analysis, Vol. II, Seventh Edition, 2007, Nirali Publication, 1-3, 28 – 30, 48, 160-164, 169.

[8]. Willard H. H., Merritt L. L., Dean J. A., Settle F. A., Instrumental Methods of Analysis, Seventh Edition, CBS Publishers and Distributors Pvt. Ltd., 118-148.

[9]. F.P. Treadwell, "Analytical Chemistry'' fourth edition, volume 1st., Published by John Wiley and Sons, INC, 1916; 1-3.

[10]. Jeffery G. H., Bassett J., Mendham J., Denney R. C., Vogel's Textbook of Quantitative Chemical Analysis, Fifth Edition, Longman Scientific and Technical Publishers, 1991, 3 – 13.

[11]. Pavia D. L., Lampman G. M., Kriz G. S., Introduction to spectroscopy, Third Edition, Thomson learning publication, 356.

[12]. Recent developments in derivative ultraviolet/visible absorption spectrophotometry by Ojeda C. B. et al., from Anal Chem Acta, 2004; 518 (1): 1 – 24.

[13]. Determination of certain drugs in multicomponent formulations by first derivative ultraviolet spectrophotometry by Korany M. A. et al., from Anal Lett, 1985; 18: 21 – 34.

[14]. Simultaneous analysis of Co2+, Cu2+, Mn2+, Ni2+ and Zn2+ in the ultraviolet region using 4 – (pyridil – 2 – azo) resorcinol and multivariate calibration by Saldanha T. C. et al., from Anal Lett., 2000; 33 (6): 1187 – 1202.

[15]. Simultaneous determination of acetaminophen and caffeine in tablet preparationsby partial least – squares multivariate spectrophotometric calibration by Bozdogan A. et al., from Talanta, 1992; 39 (8): 977 – 979.

[16]. A review on UV Spectrophotometric methods for simultaneous multicomponent analysis by Amira H. Kamal et al., from European Journal of Pharmaceutical and Medical Research, 2016; 3 (2): 348 – 360.

[17]. Spectrophotometric methods for simultaneous estimation of dexibuprofen

and paracetamol by Chitlange S. S. et al., from Asian J. Res Chem, 2009; 2 (1): 30 – 33.

[18]. Simple spectrometric analysis of propranolol hydrochloride and hydrochlorothiazide from combined pharmaceutical dosages by Erram S. V. et al., from Indian Drugs, 1994; 31: 65 – 72.

[19]. Development and validation of UV – Spectrophotometric method for the simultaneous estimation of cilnidipine and telmisartan in tablet dosage form utilising simultaneous equation and absorption ratio method by M. Haripriya et al., from International Journal of Pharmacy and Biological Science, 2013; 3 (1): 343 – 348.

[20]. Spectrophotometric estimation of cilnidipine in bulk and pharmaceutical dosage form using N – (1 – naphthyl) ethylene diamine dihydrochloride by K. S. Kokilambigai et al., from International Journal of Pharmacy and Pharmaceutical Sciences, 2014; 6 (9): 576 – 578.

[21]. Development and validation of spectrophotometric method for simultaneous estimation of azilsartan kamedoxomil and cilnidipine in synthetic mixture by Riddhi J. Jani et al., from World Journal of Pharmaceutical Research, 2018; 7 (8): 948 – 958.

[22]. Development and validation of Q – absorbance ratio spectrophotometric method for simultaneous estimation of cilnidipine and metoprolol succinate in bulk and combined dosage form by Tushar K. Kadia et al., from International Journal of Pharmacy and Pharmaceutical Sciences, 2014; 6 (6): 401 – 407.

[23]. UV – Spectrophotometric method for estimation of telmisartan in bulk and tablet dosage form by Ajit Pandey et al., from International Journal of Chem Tech Research, 2011; 3 (2): 657 – 660.

[24]. Development and validation of an UV Spectrophotometric method for simultaneous determination of cilnidipine and chlorthalidone by Snehal N. Patel et al., from Journal of Pharmacy Research, 2015; 9 (1): 41 – 45.

[25]. Spectrophotometric estimation of cilnidipine in tablets by Mohammed M. Safhi from British Journal of Pharmaceutical Research, 2015; 7 (6): 451 – 456.

[26]. Development and validation of dual wavelength UV Spectrophotometric method for simultaneous estimation of cilnidipine and olmesartan medoxomil in tablet dosage form by Isha J. Soni et al., from International Journal of Pharmaceutical and Biological Research, 2014; 2 (1): 76.

[27]. UV Spectrophotometric area under curve method for the simultaneous determination of bisoprolol fumarate and cilnidipine in pharmaceutical dosage form by Shubhada Pawar et al., from World Journal of Pharmaceutical Sciences, 2020; 9 (5): 1691 – 1699.

[28]. Development and validation of novel uv and rp – hplc method for determination of cilnidipine (a new generation ca2+ channel blocker) in pharmaceutical dosage form by P. Ravi Sankar et al., from International Journal of Pharmaceutical Sciences and Research, 2019; 10: 1886 – 1896.

[29]. Novel NP and RP – HPTLC in praxis for simultaneous estimation of chlorthalidone and cilnidipine in bulk and pharmaceutical formulation by Ravsaheb H. Rathod et al., from Analytical Chemistry Letters, 2018; 8 (6): 862 –871.

[30]. Development and validation of stability indicating RP – HPLC method for estimation of cilnidipine by Balakrishna Tiwari et al., from Journal of Drug Delivery and Therapeutics, 2020; 10 (1): 97 – 100.

[31]. Bioanalytical method development and validation for simultaneous determination of chlorthalidone and cilnidipine drugs in human plasma by RP – HPLC by M. Mukkanti Eswarudu et al., from International Journal of Research in Pharmacy and Chemistry, 2019; 9 (1): 33 – 44.

[32]. A comprehensive validation method and development of RP – HPLC for simultaneous estimation of metoprolol, telmisartan and chlorthalidone in bulk and it's formulation by V. P. Shahana et al., from Asian Journal of Research in Chemistry, 2018; 11 (6): 827 – 834.

[33]. Multivariate UV Spectrophotometric quantification of cilnidipine in bulk drug and pharmaceutical formulations by Vimal Ravi et al., from Journal of Medical

Pharmaceutical and Allied Sciences, 2022; 11 (2): 4672 – 4678.

[34]. A simple, significant UV – Spectroscopic analytical method development and validation for estimation of formulation drug product – cilnidipine tablet (oral dosage form) by Rajesh S. Jadhav et al., from International Journal of Pharmacy and Biological Sciences, 2018; 8 (3): 187 – 194.

[35]. Development and validation of analytical method for simultaneous estimation of cilnidipine and olmesartan medoxomil in bulk and tablet dosage form by HPTLC by Amit S. Minase et al., from Journal of Advanced Scientific Research, 2014; 5 (03): 34 – 38.

[36]. Simultaneous determination of cilnidipine and valsartan in synthetic mixture using spectrophotometric technique (simultaneous equation method) by Farhana V. Buchiya et al., from Asian Journal of Pharmaceutical Analysis, 2015; 5 (1): 21 –25.

[37]. Stability indicating simultaneous validation of telmisartan and cilnidipine with forced degradation behaviour study by RP – HPLC in tablet dosage form by Reema H. Rupareliya et al., from Hindawi Publishing Corporation – ISRN Chromatography, 2013; 1 – 3, https://dx.doi.org/10.1155/2013/461461

[38]. Development and validation of stability indicating gradient RP – HPLC method for simultaneous estimation of telmisartan and chlorthalidone in bulk API and fixed dose combination by Bhamini R. Chaudhary et al., from World Journal of Pharmaceutical Research, 2017; 6 (10): 1015 – 1029.

[39]. The Indian Pharmacopoeia, Government of India, Ministry of Health & Family Welfare, 2018; vol/II: 1616 – 1617.

[40]. National Center for Biotechnology Information, 2021. PubChem Compound Summary for CID 5282138, Cilnidipine.

[41]. The Indian Pharmacopoeia, Government of India, Ministry of Health & Family Welfare, 2014; vol/III: 2830 – 2831.

[42]. National Center for Biotechnology Information,2022. PubChem Compound Summary for CID 65999, Telmisartan.

[43]. Ultraviolet Spectroscopy and it's Pharmaceutical Applications – A Brief Review by Dipali M. Atole et al., from Asian Journal of Pharmaceutical and Clinical Research, 2018; 11 (2): 59 – 66.

[44]. ICH Harmonised Tripartite Guideline (2005) Validation of Analytical Procedures: Text and Methodology Q2 (R1). International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Geneva, 1-13.