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Dual Wavelength Spectrophotometric Method for Estimation of Cilnidipine and Telmisartan in Their Combined Dosage Form.

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Research Article

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ABSTRACT

The study aim to develop simple, sensitive, rapid, accurate and precise spectrophotometric method for estimation on Cilnidipine (CIL) and Telmisartan (TEL) in combined dosage forms. Pure drug sample of Cilnidipine and Telmisartanwere dissolved in a methanol. From the overlain spectra, four wavelengths 264 nm (λ1), 297.4 nm (λ2), 229 nm (λ3)and 246.8 nm (λ4) were selected for quantitation of both the drugs by proposed Dual wavelength spectrophotometric method. The quantitative determination of CIL was carried out by measuring the absorbance difference at λmax of 264 nm and 297.4 nm where TEL shows same absorbance value. The quantitative determination of TEL was carried out by measuring the absorbance difference at 229 nm and 246.8 nm where CIL showed same absorbance value at both the wavelengths. The results of analysis were validated statistically and by recovery studies and found to be free from interferences. These methods obey Beer's law in the concentration range 2-6 µg/ml and 3-15 µg/ml for CILand TELrespectively.

INTRODUCTION

Cilnidipine (CIL) is a light yellowish powder. Chemically it is 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid2-methoxyethyl(2E)-3phenyl-2-prpenyl ester [1](Fig.1.A). It is antihypertensive agent and calcium channel blocker. Cilnidipine is a dual L-/N-type calcium channel protein inhibitor and blocker. Cilnidipine has displayed renal andvascular protective effects and improved baroreflexsensitivity in patients with hypertension [1,2,3,4,5]. Telmisartan (TEL) is white crystalline powder. Chemically, it is 4 ′ -[[4-Methyl-6-(1-methyl-1H-benzimidazol-2-yl)-2-propyl-1H-benzimidazol-1-yl]methyl]biphenyl-2-carboxylic acid[6,7,8] (Fig. 1: B). It is very soluble in methanol and practically insoluble in water. It isAngiotensin-converting Enzyme Inhibitors and Angiotensin II Type 1 Receptor Blockersagents. The mechanism by which Telmisartan is an angiotensin II receptor blocker (ARB)that shows high affinity for the angiotensin II receptor type 1 (AT1), with a binding affinity3000 times greater for AT1 than AT2. It has the longest half-life of any ARB (24 hours) and the largest volume of distribution. The combination of CIL and TEL is indicated asantihypertensive agents.[8] Literature survey revealed that Cilnidipine can be estimated by spectrophotometry [10, 11] and by liquid chromatographic methods [12,13,14] individually or in combination with other drugs, and Telmisartan can be estimated by spectrophotometry[15,16,17,18,19,20,21] and by liquid chromatographic methods individually or in combination with other drugs [22,23,24] . Two methods UV spectroscopy [25, 26] have been reported for the estimation of Cilnidipine and Telmisartan in their combined dosage form. Dual wavelength spectrophotometric method is considered to be a good alternative, and it should be widely explored as an important tool in routine drug analysis. The aim of the present work was to develop an accurate, repeatable, sensitive and specific UV spectrophotometric method for the determination of CIL and TEL in formulation as stipulated by the ICH guidelines. The proposed method was validated according to ICH guidelines and its updated international convention.

MATERIALS AND METHODS

Instruments

A Shimadzu UV-1800, UV/Visible spectrophotometer with spectral band width of 1nm, wavelength accuracy of ± 0.3 nm and 1 cm matched quartz cells was used for analytical method development.

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Preparation of standard stock solution (100µg/ml)

Accurately weighed portion of CIL and TEL 10 mg was transferred to a 10 ml volumetric flask and dissolved and diluted to the mark with methanol to obtain solution having concentration of CIL and TEL (1000 µg/ml). From this 1 ml was pipetted out in 10 ml volumetric flask and diluted to the mark with methanol to obtain standard stock solution of 100 µg/ml.

Selection of analytical wavelength

Accurately weighed 10 mg of CIL and 10 mg TEL was transferred into 100 ml volumetricflask, separately and dissolved in small volume of methanol. The volume was adjusted to the mark with methanol to obtain final concentration of CIL and TEL (100 µg/ml). 1 ml of this solution was transferred in 10 ml volumetric flask and volume was adjusted to the mark with methanol, to prepare a final concentration 10 µ g/ml. This standard solution of CIL and TEL was scanned in UV range 200-400 nm in 1cm cell against methanol was blank and maximum absorbance was measured for selection of λ max of CIL and TEL.From the overlain spectra, four wavelengths 264 nm (λ1), 297.4 nm (λ2), 229 nm (λ3)and 246.8 nm (λ4) were selected for quantitation of both the drugs by proposed Dual wavelength spectrophotometric method. The quantitative determination of CIL was carried out by measuring the absorbance difference at λmax of 264 nm and 297.4 nm where TEL shows same absorbance value . The quantitative determination of TEL was carried out by measuring the absorbance difference at 229 nm and 246.8 nm where CIL showed same absorbance value at both the wavelengths. Hence, the absorbance difference between 229 nm and 246.8 nm was directly proportional to concentration of TEL.

Calibration curve for CIL and TEL

Appropriate aliquot of stock solution was taken in five different 10 ml volumetric flask.Volume was made up to the mark with methanol to obtain final concentration of 2, 3, 4, 5, 6g/ml of CIL and 3,6,9,12,15 µg/ml of TEL respectively.

Estimation of CIL and TEL in Tablet Dosage Form

Twenty tablets (label claim of 10 mg CIL and 40 mg TEL) were weighed and finely powered. Powder equivalent to 10 mg CIL and 40 mg TEL was accurately weighed and transferred to 100 ml volumetric flask addition of methanol and sonicated for 15 min. The volume was made up to mark with methanol. The solution was filtered through whatmann filter paper no 41. From this solution, 1 ml was transferred to 10 ml volumetric flask and volume give a solution containing 10 µg/ml CIL and 40 µg/ml TEL. This solution was used for the estimation of CIL and TEL. The responses of the sample solution were measured at 264 nm (λ1), 297.4 nm (λ2), 229 nm (λ3) and 246.8 nm (λ4) for quantification of CIL and TEL respectively. The amount of CIL and TEL present in the sample solution were determined by substituting the absorbance into the regression equation for CIL and TEL respectively.

VALIDATION OF UV METHOD [27]

Linearity and Range

The linearity was evaluated through a linear regression analysis. The linearity for CIL (2– 6 µg/ml) at 264 nm (λ1), 297.4 nm (λ2) and TEL (3 -15 µ g/ml) 229 nm (λ3) and 246.8 nm (λ4) at was determined in terms of correlation coefficient.

Accuracy

To demonstrate the accuracy of the proposed method, recovery studies were carried out by standard addition method. Solution of formulation in concentration 2 µg/ml for CIL and 3µg/ml for TEL was spiked with 50%, 100% and 150% concentration of standard for CIL (1, 2,3 µg/ml) and for TEL(1.5,3,4.5 µg/ml) respectively. % recovery was then calculated by using regression equation.

Precision

Repeatability

Standard solutions of CIL (2, 3, 4, 5, 6 µg/ml) and of TEL(3, 6, 9, 12, 15 µg/ml) were prepared and spectras were recorded. Absorbance was measured at 252 nm using methanol as a blank. The absorbances of the same concentration solution were measured six times and %RSD was calculated.

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Intra and inter day precision

Variation of results of three different concentrations (2, 4 and 6 µg/ml) of CIL and (3, 9, 15 µg/ml) of TEL within the same day (intra- day) & variation of results between different days (inter- day) were analyzed.Intra-day precision was determined by analyzing CIL and TEL for three times in the same day.

Inter-day precision was determined by analyzing CIL and TEL daily for three days.

Limit of detection

From the linearity curve equation, the standard deviation (SD) of the intercepts (response) was calculated. Then LOD was measured by using mathematical expressions given in section.The limit of detection (LOD) of the drug was calculated by using the following equations designated by International Conference on Harmonization (ICH) guideline:

$$LOD = 3.3 * \sigma/S,$$

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

Limit of quantification

From the linearity curve equation, the standard deviation (SD) of the intercepts (response) was calculated. Then LOQ was measured by using mathematical expressions given in section.The limit of quantification (LOQ) of the drug was calculated by using the following equations designated by International Conference on Harmonization (ICH) guideline:

$$LOQ = 10 * \sigma/S$$

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

Solution stability

The sample preparations were analyzed by UV at regular intervals for 24 hrs as per test procedure.

RESULT AND DISCUSSION OF UV METHOD

Selection of solvent

CIL and TEL are soluble in Methanol but it is insoluble in Water. So spectrum of CIL and TEL was scanned in Methanol.

Selection of analytical wavelength

Dual wavelength method was used for estimation of CIL and TEL; In this method, two wavelengths are required for one drug where one drug shows similar absorbance but other drug shows difference in absorbance. Two wavelengths were selected at which CIL showed similar absorbance while other drug TEL showed considerable difference in absorbance. The other two wavelengths were selected such that TEL showed similar absorbance while CIL showed considerable difference in absorbance. The overlay spectrum of CIL and TEL at different concentrations revealed that at 229 nm and 246.8 nm different concentrations of CIL showed similar absorbance whereas TEL showed significant difference in the absorbance. In a similar manner, at 264 nm and 297.4 nm different concentrations of TELshowed similar absorbance whereas CIL showed significant difference in absorbance. Considering above facts, wavelength 264 nm and 297.4 nm were selected for the estimation of CIL while 229 nm and 246.8 nm were selected for the estimation of TELShown in Figure 4.1 and 4.2

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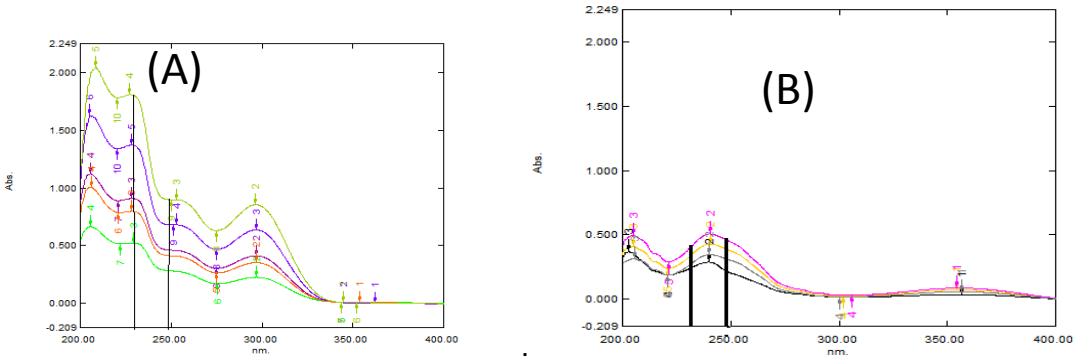


- Figure 1: (A),(B) Spectra of CIL and TEL for different concentration at 229 nm& 246.8 nm Where CIL showed same absorbance and TEL showed difference in absorbance

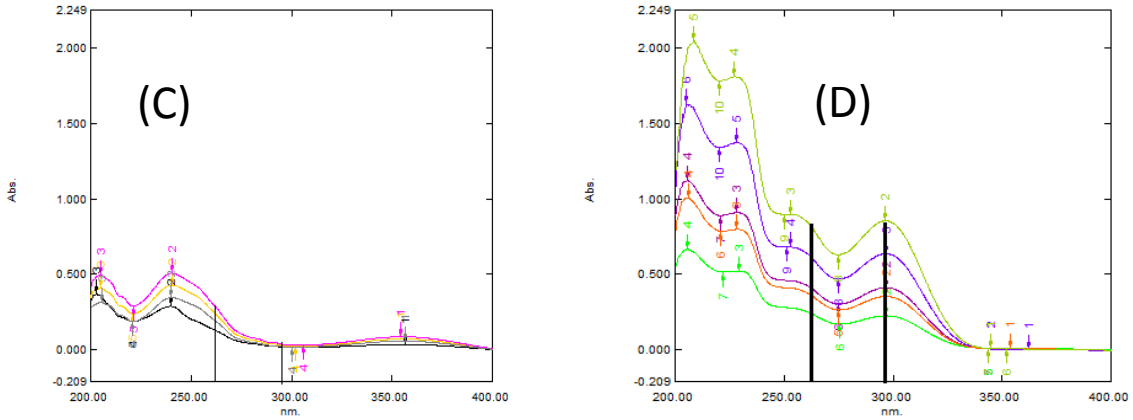
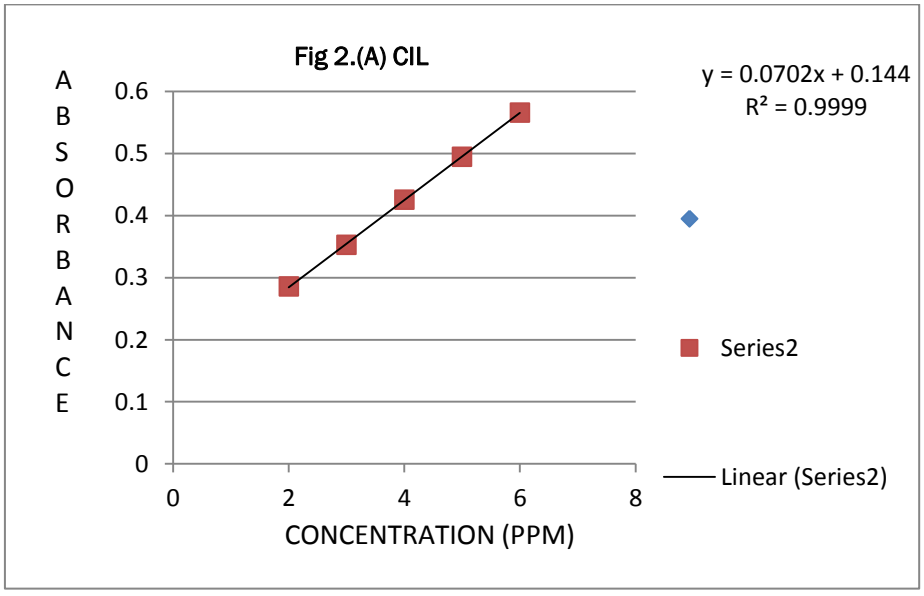


Figure 2: (C),(D) Spectra of CIL and TEL for different concentration at 264 nm & 297.4 nm Where TEL showed same absorbance and CIL showed difference in absorbance

VALIDATION OF DEVELOPED UV METHOD

Linearity and Range

The calibration curve when plotted was found to be linear over the concentration range of 2-6 µg/ml for CIL and 3-15 µg/ml The calibration curve when plotted was found to be linear over the concentration range of 2-6 µg/ml for CIL and 3-15 µg/ml for TEL with regression coefficient (r2) 0.999 and 0.996 respectively. Calibration curves for CIL and TEL were constructed by plotting difference inabsorbance VS concentration.(Figure 2 (A) , Figure 2 (B).The developed Spectrophotometry method was validated. The linear range, correlation coefficient, detection limit and standard deviation for CIL and TEL by Spectrophotometry method are shown in Table 1.



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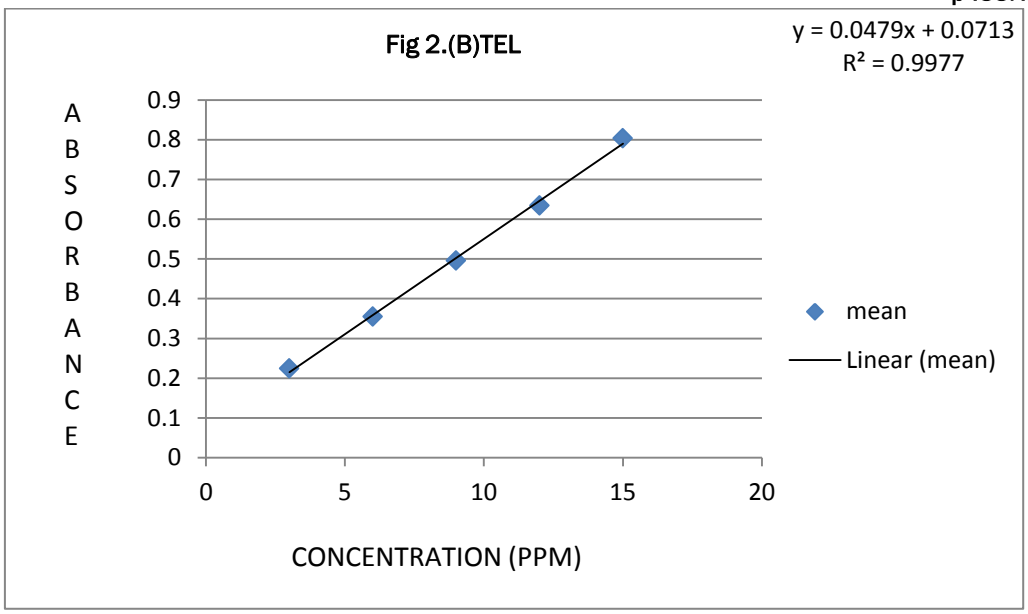

Table 1: Result of calibration readings for CIL and TEL

Concentration (ppm) CIL	Absorbance Diff of CIL at 264 and 297.4 nm Mean ± SD	%RSD	Concentration (ppm) TEL	Absorbance of TEL at 229 and 246.8 nm Mean ± SD	%RSD
2	0.285 ±0.0019	0.84	3	0.224±0.0023	0.59
3	0.352 ±0.0057	0.73	6	0.354±0.0051	0.54
4	0.425±0.064	0.48	9	0.495±0.04	0.59
5	0.494±0.0077	0.48	12	0.634±0.0087	0.30
6	0.565±0.0073	0.42	15	0.803±0.0033	0.14

Accuracy

Accuracy was determined by calculating the recovery. The method was found to be accurate with percent recovery 99.7 – 101.89 % and 99.3-101.3 % for CIL and TEL respectively (Table 2 and 3).

Table 2: Determination of Accuracy for CIL

Accuracy %	Amount of drug from formulation (µg/ml)	Amount of standard drug added (µg/ml)	Amount of drug found (µg/ml)	% Recovery	%RSD
0	2	0	2.03±0.45	100.6	1.08
50	2	1	2.8±0.74	92.2	1.60
100	2	2	4.01±0.34	99.6	1.09
150	2	3	5.01±0.19	99.1	0.49

Table 3: Determination of Accuracy for TEL

Accuracy %	Amount of drug from formulation (µg/ml)	Amount of standard drug added (µg/ml)	Amount of drug found (µg/ml)	% Recovery	%RSD
0	3	0	3.04±0.40	100.3	1.06
50	3	1.5	4.5±0.47	100.8	0.69
100	3	3	6.04±0.37	101.6	0.68
150	3	4.5	7.5±0.13	99.85	1.06

Precision

Repeatability

Repeatability data are shown in (Table4) .The % RSD is < 2 for CIL and TEL respectivelywhich indicate that the method is precise.

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Table 4: Repeatability data for CIL and TEL at 250 nm and 297nm

Concentration	CIL 4 µg/ml (n = 6)	TEL 9 µg/ml (n = 6)
Absorbance	0.428	0.499
	0.425	0.493
	0.425	0.498
	0.427	0.493
	0.423	0.495
	0.423	0.493
Mean.	0.425	0.496
Std. Dev.	0.0020	0.0029
% RSD	0.48	0.59

Intra and inter day precision

Variation of results within the same day (intra- day), variation of results between days (interday) were analyzed. For intra-day (n=3) % RSD was found to be 0.17-0.52 and 0.12-0.44 CIL and TEL respectively and % RSD for inter-day (n=3) was 0.77-0.89 and 0.12-1.18 for CIL and TEL respectively (Table 5). The % RSD is < 2 for both the drugs which indicate that the method is precise.

Table 5: Precision data for CIL and TEL at 250 nm and 297 nm

Concentration (ppm) CIL	Precision (% RSD) (n=3)		Concentration (ppm) TEL	Precision (% RSD) (n=3)	
	Intraday	Interday		Intraday	Interday
2	0.53	0.89	3	0.4	1.18
4	0.36	0.84	9	0.23	0.61
6	0.17	0.77	15	0.12	0.12

Limit of detection and limit of quantification

Under the experimental conditions used, the lowest amount of drug that could be detected (LOD) for CIL and TEL was found to be 0.05 µg/ml and 0.088 µg/ml respectively. The limit of quantification (LOQ) for CIL and TEL was found to be 0.16 µg/ml and 0.266 µg/ml respectively, with an RSD < 2%.

Solution stability

The sample preparations were analyzed regular intervals for 24 hrs as per test procedure. The method is also rugged as there was no change in absorbance up to 24 hours of preparation of solution in Methanol (Table 6).

Table 6: Solution Stability Study

Time (hours)	Absorbance of CIL(2µg/ml)(n=3)	Result (%)	Absorbance of TEL (3µg/ml)(n=3)	Result (%)
0	0.285±1.27	100.00	0.226 ± 1.04	100.01
4.0	0.279±1.89	99.30	0.223 ± 1.22	99.4
8.0	0.274±0.74	99.07	0.220 ± 0.23	99.1
24.0	0.281±0.67	99.54	0.224 ± 0.75	99.79

Ruggedness

Ruggedness of the method was checked by different parameters like solvent of different manufacturer, analyst and UV-Visible spectrophotometer model (Table 7).

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Table 7: Data of ruggedness of method

Conc. (µg/ml)		Change in Condition	Avg. absorption ± SD		%RSD	
CIL	TEL		CIL	TEL	CIL	TEL
2	3	SRL*	0.286±0.007	0.229 ±0.005	1.63	1.45
		Rankem*	0.285±0.004	0.228 ±0.005	0.9	0.67
		1#	0.285±0.004	0.228 ±0.005	0.78	0.74
		2#	0.284±0.007	0.229 ±0.005	1.08	1.11
		UV 1700@	0.285	0.228 ±0.005	1.61	1.28
		UV 1800@	0.284	0.229 ±0.005	1.74	1.53

* Solvent of different manufacturer, # Analyst, @ UV-Vis Spectrophotometer model

Table 8: Summary of validation parameters

Parameters	CIL	TEL
Range (ppm)	2-6	3-15
Detection limit (ppm)	0.05	0.088
Quantitation limit (ppm)	0.16	0.266
Accuracy (%)	99.7-101.8	99.3-101.3
Precision (%RSD)		
Intra-day precision (n=3)	0.17-0.53	0.12-0.44
Inter-day precision (n=3)	0.77-0.89	0.12-1.18
Repeatability study (n = 6)	0.48	0.59

Marketed formulation was analyzed by the proposed method and assay result of marketedformulation is shown in Table 9

Table 9: Assay Results of Marketed Dosage Forms

Formulations	Labeled Amount (mg)	% Recovery (n=3)
Cilacar T	10 mg CIL and 40 mg TEL	99.39-101.89

CONCLUSION

In UV- Spectrophotometric method, correlation coefficient was found to be 0.999 and 0.997 for CIL and TEL respectively. Linear range was found to be 2-6 µg/ml and 3-15 µg/ml for CIL and TEL respectively.

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