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DEVELOPMENT AND VALIDATION OF UV-VISIBLE SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF CILNIDIPINE AND TELMISARTAN IN BULK AND DOSAGE FORM

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ABSTRACT

Simple, rapid, sensitive, precise and specific UV spectrophotometric method for the determination of Cilnidipine (CIL) and Telmisartan (TEL) in bulk drug and pharmaceutical dosage form were developed and validated**.** A simple double beam UV spectrophotometric method has been developed and validated with different parameters such as linearity, precision, repeatability, limit of detection (LOD), Limit of Quantification (LOQ), accuracy as per ICH guidelines. UV-visible spectrophotometric method, measurement of absorption at maximum wavelength in 10 ml acetonitrile and volume make with water solvent system as reference CIL and TEL were found to be at 203 nm and 241nm respectively. The drug obeyed the Beer's law and showed good correlation. Beer's law was obeyed in concentration range 0.5-2.5 µg/ml for Cilnidipine and 2-10µg/ml for Telmisartan respectively with correlation coefficient was 0.999. The LOD and LOQ of CIL were found to be 0.317 (µg/ml) and 0.96 (µg/ml), TEL were found to be 0.67 (µg/ml) and 5.086 (µg/ml), respectively. Percentage assay of CIL and TEL in tablets. The proposed method is precise, accurate and reproducible and can be used for routine analysis of CIL and TEL in bulk and pharmaceutical dosage form.

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INTRODUCTION

Cilnidipine (CIL) O3-(2-methoxyethyl) O5-[(E)-3- phenylprop-2-enyl] 2, 6-dimethyl-4-(3- nitrophenyl)-1,4-dihydropyridine-3,5- dicarboxylate is a novel and unique dihydropyridine calcium channel blocker that possesses a slow-onset, long-lasting vasodilating effect. It is not official in any Pharmacopoeia. Telmisartan (TEL), 4-((2-n-propyl-4-methyl-6-(1- methylbenzimidazol – 2 – yl) – benzimidazol – 1 - yl) methyl) biphenyl-2- carboxylic acid, 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 for AT1 than AT2 (Figure 1). It is official in I. P. and B. P. [1-7]

Literature survey reveals that several methods such as High Performance Liquid Chromatography, UV spectrophotometry, HPTLC etc. [8-28] As there is no analytical method reported for quantitative estimation of CIL and TEL in combination But the present study is to develop an accurate and reliable UV visible spectrophotometric method for simultaneous estimation of CIL and TEL in tablet dosage form as per ICH norm.

MATERIALS AND METHODS

Reagents and Materials

A Shimadzu UV/Visible double beam spectrophotometer (Model 1700) with 1cm matched quartz cells was used in present study for multi component analysis. CIL and TEL in the form of gift samples were kindly supplied by R. S. I .T. C, Jalgaon respectively. AR grade methanol used for UV method and 0.1N HCl and pH 6.8 phosphate buffer were prepared in double distilled water was used as solvent throughout the study. A combination of CIL (10 mg) and TEL (40 mg) in tablet formulation was procured from local pharmacy (CILACAR- T, J.B Chemical Pharmaceutical Pvt. Ltd.

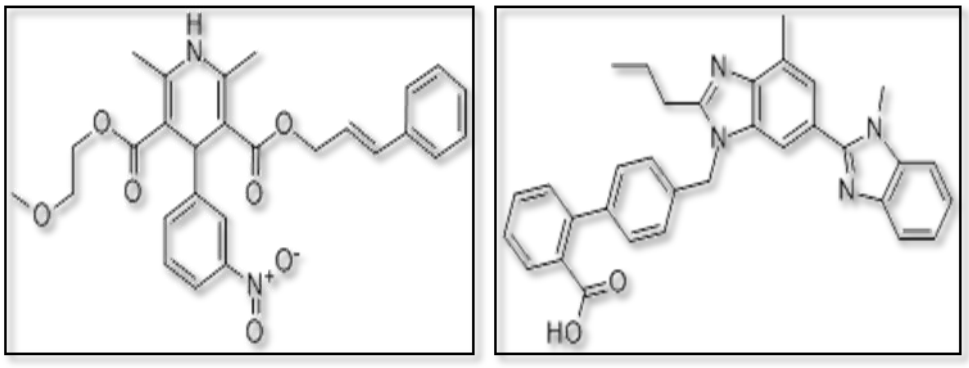

Fig. 1: Structure of Cilnidipine and Telmisartan.

Preparation of standard stock solution

Accurately weight and transfer 10 mg Cilnidipine and Telmisartan 40 mg working standard into 10 ml volumetric flask as about diluents Acetonitrile completely and make volume up to the mark with the same solvent to get 1000µg/ml standard (stock solution) and 15 min sonicate to dissolve it and remove the unwanted gas, further an aliquots portion of CIL and TEL stock solution in ratio of 70:30were mixed in volumetric flask in 10 ml and volume was adjusted up to mark with mobile phase from the resulting solution 0.1ml was transferred to 10 ml volumetric flask and the volume was made up to the mark with Acetonitrile : Pot. Phosphate Buffer, prepared in (7ml Acetonitrile: 3ml Pot. Phosphate Buffer) solvent. Result was shown as; (Figure 2, 3 and 4).

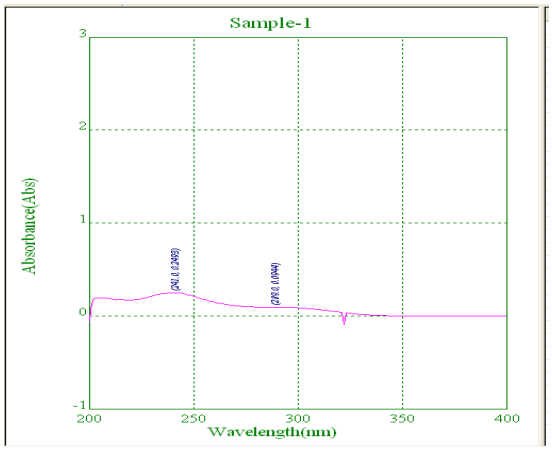

Figure 2: UV Spectrum of Cilnidipine

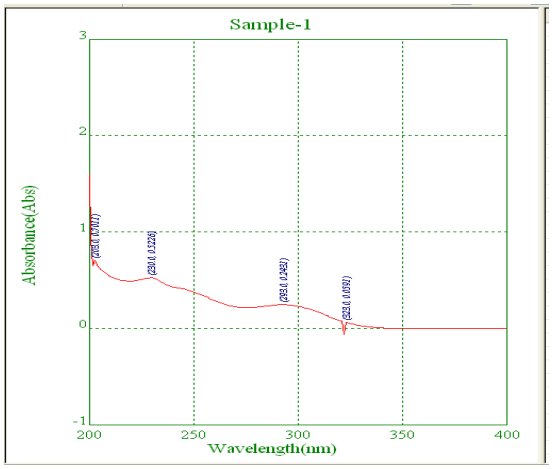

Figure 3: UV Spectrum of Telmisartan.

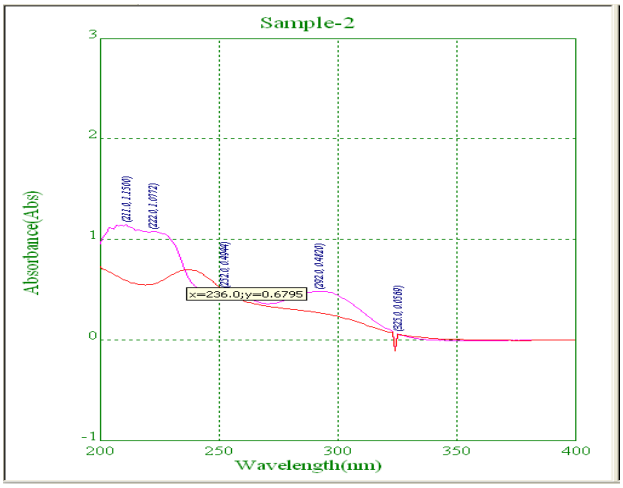

Figure 4: Iso-absorptive point of Cilnidipine and Telmisartan.

Procedure for calibration curve of Cilnidipine and Telmisartan:

The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. From the freshly prepared standard stock solution, pipette out 10mg Cilnidipine and 40mg Telmisartan in 10ml of volumetric flask and diluted with mobile phase. From it 0.1, 0.2, 0.3, 0.4 and 0.5ml of solution were pipette out in 10 ml volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 5, 10, 15, 20, 25 µg/ml of Cilnidipine and 20, 40, 60, 80,100µg/ml of Telmisartan (Table 1 and 2). The respective linear equation for CIL was y = 0.059x + 0.081 and TEL equation y = 0.070x + 0.045 where x is the concentration and y is area of peak. The correlation coefficient was 0.999. The calibration curve of CIL and TEL recorded at 236 nm as the graph plotted as concentration of drug verses peak area is depicted in **(**Fig. 5 and 6) respectively. [29-30].

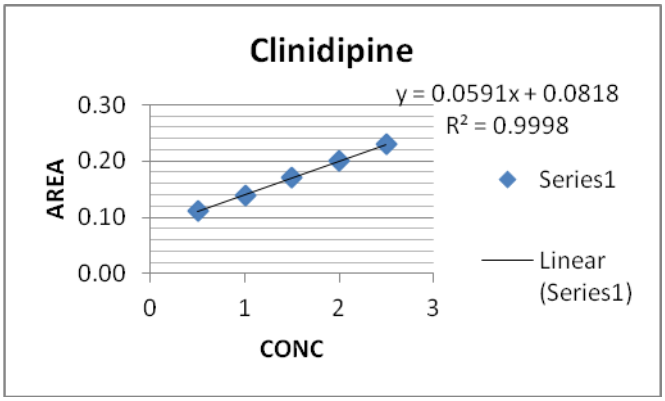

Figure 5: Calibration curve of Cilinidipine.

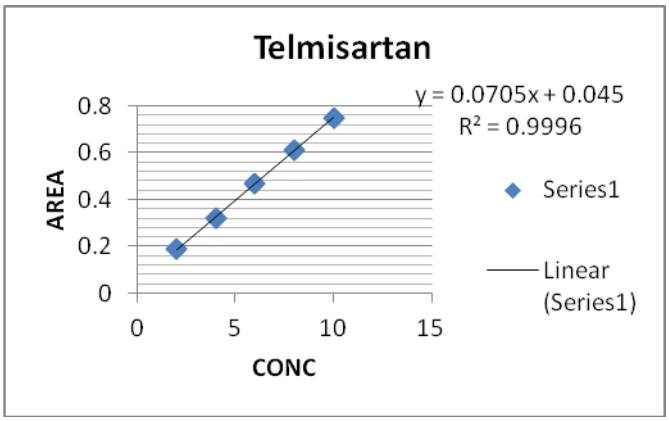

Figure 6: Calibration curve of Telmisartan.

Table 1: Linearity data for Cilnidipine.

Method	Conc. µg/ml	Peak area(µV.sec) 1	2	Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
UV Method	0.5	0.107	0.108	0.11	0.00	0.66
	1	0.134	0.136	0.14	0.00	1.05
	1.5	0.17	0.174	0.17	0.00	1.64
	2	0.201	0.20	0.20	0.00	0.35
	2.5	0.22	0.221	0.22	0.00	0.32
	Equation		Y = 0.059x+0.081			
		R^2	0.999			

Table 2: Linearity data for Telmisartan.

Method	Conc. µg/ml	Peak area (µV.sec) 1	2	Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
UV Method	2	0.192	0.1921	0.19	0.00	0.04
	4	0.31	0.32	0.32	0.01	1.24
	6	0.48	0.47	0.48	0.01	1.49
	8	0.61	0.62	0.99	0.01	0.71
	10	0.75	0.74	0.75	0.01	0.95
	Equation		y = 0.070x+0.045			
		R^2	0.999			

Selection of detection wavelength :

Standard solutions were scanned in the range of 200-400 nm, against 10 ml acetonitrile and volume make with water solvent system as reference CIL (Figure 2) and TEL (Figure 3) were showed absorbance maxima (lamda max) at 203nm and 241nm respectively (Fig. 4). If Two CIL and TEL sample Interact with this point is called isobestic point. The detection of wavelength in isobestic point in 236 nm.

Procedure for analysis of tablet formulation

Weigh 20 Cilnidipine and Telmisartan combination tablets and calculated the average weight, accurately weigh and transfer the sample equivalent to 364 mg CIL and TEL into 10 ml volumetric flask. Add about 10ml ACN of diluents and sonicate to dissolve it completely and make volume up to the mark with diluents. Mix well and filter through 0.45 µm filter. Further pipette 0.4ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents (40 µg/ml). The simple chromatograms of test CIL and TEL shown in (Figure 7). The amounts of CIL and TEL per tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with tablet formulation. Tablet Assay for %Label claim for % RSD Calculated, Result was shown in **(**Table 3).

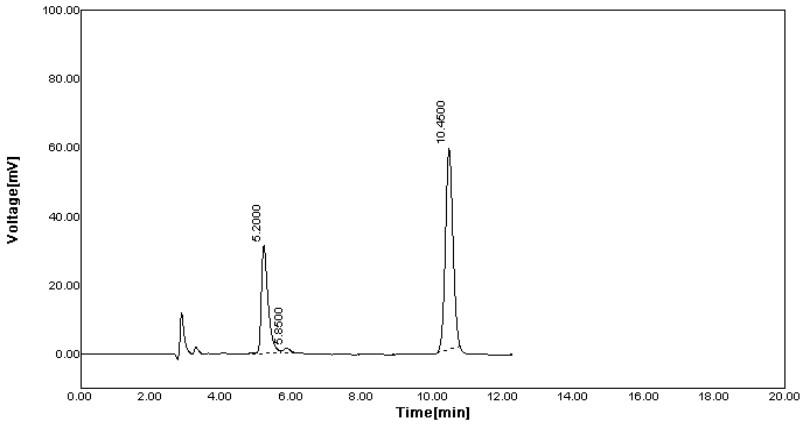

Figure 7: Chromatogram for marketed formulation.

Table 3: Analysis of marketed formulation.

Assay	Drug	Label Claimed	Amt. Found	%Label Claim	SD	%RSD
UV Method	CILIN	25	1.01	101.35	0.01	0.02
	TEL	100	4.08	102.00	0.01	0.17
	CILIN	25	1.02	101.86	0.35	0.37
	TEL	100	4.09	102.25	0.64	0.61

METHOD VALIDATION

The proposed methods were validated accordance to ICHQ2 (R1) guidelines for linearity, precision, accuracy, limit of detection, limit of quantification.

RESULTS

Linearity and Range:

The linearity of proposed methods were evaluated by linear regression analysis, which was calculated by least square method. Calibration standards were prepared by spiking required volume of working standard solution10mg CIL and 40mg TEL in 10ml of volumetric flask and diluted with mobile phase. From it 0.1, 0.2, 0.3, 0.4 and 0.5ml of solution were pipette out in 10 ml volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 5, 10, 15, 20, 25 µg/ml of Cilnidipine and 20, 40, 60, 80, 100µg/ml of Telmisartan (Table 1 and 2). Absorbance of the drugs was measured. Calibration curve was plotted between absorbance of drug against concentration of the drug. These results shown there was an excellent correlation between absorbance and analyte concentration of drug verses peak area is depicted in (Fig. 5 and 6) respectively.

Accuracy:

Accuracy of the methods was determined at three different concentration levels i.e.80%, 100% and 120% in triplicate for each drug as per ICH guidelines. From the total amount of drug found, the percentage recovery was fond in range of 99-101% (Table 4 and 5).

Table 4: Results of recovery data for Cilnidipine and Telmisartan.

Method	Drug	Level (%)	Amt. taken (µg/ml	Amt. Added (µg/ml	Absorbance Mean*± S.D.	Amt. recovered Mean*± S. D.	%Recovery Mean *± S.D.
UV Method	CILN	80%	1	0.8	1.79±0.01	0.79±0.01	99.26±1.05
		100%	1	1	2.03±0.01	1.03±0.01	103.05±0.71
		120%	1	1.2	2.21±0.02	1.21±0.02	100.56±1.60
	TEL	80%	4	3.2	7.23±0.01	3.23±0.01	100.65±0.91
		100%	4	4	8.08±0.01	4.08±0.01	102.03±0.20
		120%	4	4.8	8.81±0.01	4.81±0.00	100.34±0.06

*mean of each 3 reading for UV method.

Table 5: Statistical validation of recovery studies Cilnidipine and Telmisartan.

Method	Level of Recovery (%)	Drug	Mean % Recovery	Standard Deviation*	% RSD
UV Method	80%	CILIN	99.26	1.05	1.06
		TEL	100.65	0.91	0.91
	100%	CILIN	103.05	0.71	0.69
		TEL	102.03	0.20	0.19
	120%	CILIN	100.56	1.60	1.59
		TEL	100.34	0.06	0.06

*Denotes average of three determinations for UV method.

Precision:

Precision was studied to find out intra and inter-day variations in the test method of CIL and TEL. Intra-day precision was determined by analyzing three concentration in three replicate measurements of within linearity range of drugs on three different times in the same day. Inter-day precision was conducted during routine operation of the system over a period of 3 consecutive days. Intraday and Inter day Precision studies on UV method for CIL and TEL which shows the high precision % amount in between 98% to 100% indicates to analytical method that concluded (Table 6).

Table 6: Result of Intraday and Inter day Precision studies on UV method for CIL and TEL.

METHOD	Drug	Conc. (µg/ml)	Intraday Precision		Inter-day Precision	
			Mean± SD	%Amt Found	Mean± SD	%Amt Found
UV METHOD	CILIN	1	0.14±0.00	100.00	0.14±0.00	100.00
		1.5	0.17±0.00	100.50	0.17±0.00	100.50
		2	0.19±0.00	100.50	0.19±0.00	100.50
		4	0.34±0.02	101.78	0.32±0.00	101.78
		6	0.48±0.00	101.83	0.48±0.00	101.03
	TEL	8	0.63±0.00	102.63	0.62±0.00	102.63

*Mean of each 3 reading for UV method.

Limit of detection (LOD) and Limit of quantification (LOQ):

LOD is the lowest amount of analyte in a sample that can be detected but not necessarily quantify under the stated experimental conditions. LOQ is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions.

Repeatability:

Repeatability studies on UV method for CIL and TEL was found to be, the % RSD was less than 2%, which shows high percentage amount found in between 98% to 102% indicates the analytical method that concluded **(**Table 7).

Table 7: Repeatability studies on UV method for Cilnidipine and Telmisartan.

Method	Concentration of Cilnidipine and Telmisartan (mg/ml)	Peak area	Amount found (mg)	% Amount found
UV Method for CILIN	2	0.2012	2.04	101.86
	2	0.2016	2.04	102.20
	2	0.2011	2.03	101.77
	2	0.2019	2.05	102.45
	2	0.2023	2.05	102.79
		Mean	2.04	102.21
		SD	0.01	0.42
		%RSD	0.41	0.41
UV Method for TEL	8	0.621	8.22	102.85
	8	0.6105	8.07	101.41
	8	0.6215	8.23	102.30
	8	0.6236	8.29	103.62
	8	0.6279	8.32	104.08
		Mean	8.23	102.85
		SD	0.10	1.06
		%RSD	1.17	1.03

DISCUSSION

The proposed methods for simultaneous estimation of CIL and TEL in tablet dosage forms were found to be simple, accurate, economical and rapid. The method was validated as per the ICH Q2 (R1) guidelines. Standard calibration curves for CIL and TEL were linear with correlation coefficients (r2) values in the range of 99.26 – 100.65 at all the selected wavelengths and the values were average of three readings. The values of % RSD are within the prescribed limit of 2 %, showing high precision of methods and recovery was close to 100% for both the drugs. Results of the analysis of pharmaceutical formulations reveal that the proposed methods are suitable for their simultaneous determination with virtually no interference of usual additive present in pharmaceutical formulations. Hence, the above methods can be applied successfully for simultaneous estimation of CIL and TEL in formulations.

The proposed method utilize two medium i.e. 0.1 N HCL and pH 6.8 phosphate buffer. The comparison of method with already published two methods shows that the developed method is more accurate and economic as compared to other two method further the method complies with detection of drugs as per their label claim also no further derivetization or modification in spectra is required so the proposed method can be said as simple accurate and economic as compared to other published method.

CONCLUSION

The developed UV methods were found to be more accurate, precise and reproducible. The analysis of tablets containing two drugs gave the satisfactory results. The statistical parameter of these methods showed good results. The recovery studies revealed excellent accuracy and high precision of the method. The methods were found to be simple & time saving. All proposed methods could be applied for routine analysis in quality control laboratories.

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