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Method Development and Validation for Simultaneous Estimation of Telmisartan and Cilostazol

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ABSTRACT

A simple, rapid and sensitive first-order derivative method was developed for simultaneous estimation of Telmisartan and Cilostezol with detection wavelength 256 nm and 296 nm respectively. The linearity for Telmisartan was found to be 2 – 14 µg/ml and for Cilostezol2-14 µg/ml was determined in terms of a correlation coefficient. Limit of detection was found to be 0.041µg/ml and 0.01225µg/ml and the limit of quantification was found to be 0.125µg/ml and 0.037121µg/ml for Telmisartan and Cilostazol respectively. The % recovery of Telmisartan was found to be 98.53% -99.14% and 97.63% -99.1 % for Cilostazol. For repeatability % RSD was found to be 0.4 for Telmisartan and 0.3 for Cilostazol. The specificity of the method was ascertained by analyzing the standard drug and sample. The proposed method can be effectively applied for simultaneous estimation of both the drugs.

INTRODUCTION

Diabetes Mellitus is a condition where the cells of the body cannot utilize glucose properly. In type 1 there is reduced insulin production as the beta cells are gradually destroyed and increased peripheral resistance in the uptake of insulin. In type 2, the body produces enough insulin; however, the cells develop a condition called ‘insulin resistance' where glucose does not move into the cells. The body breaks down fats, proteins, and stored glycogen to produce glucose resulting in high levels of glucose in the blood and excess byproducts such as ketones, which are products of incomplete fat metabolism. (1)

Nowhere is the diabetes epidemic more pronounced than in India as the World Health Organization (WHO) reports show that 32 million people had diabetes in the year 2000. The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 40.9 million in India and this is further set to rise to 69.9 million by the year 2025. (2)

Multi-particulate drug delivery systems are more preferred as compare to conventional treatment for diabetes. To deliver the recommended total dose, these subunits are filled into a sachet and encapsulated or compressed into a tablet. They provide many advantages over single-unit systems because of their small size. Combination of Telmisartan and Cilostazol can be used for the same. (3)

Chemical name of Telmisartan (TEL) is 4'-((1,4'-dimethyl-2'-propyl(2,6'-bi-1H-benzimidazol)-1'-yl)methyl)-(1,1'-biphenyl)-2-carboxylic acid. The category of TEL is Angiotensin II receptor blockers (ARBs). The mechanism of action of TEL is it interferes with the binding of angiotensin II to the angiotensin II AT_1-receptor by binding reversibly and selectively to the receptors in vascular smooth muscle and the adrenal gland. This suggests that TEL can improve carbohydrate and lipid metabolism, as well as control insulin resistance without causing the side effects that are associated with full PPARγ activators. (4)

The chemical name of Cilostazol (CILO) is 3, 4-dihydro-6-(4-(1-cyclohexyl-1H-tetrazole-5-yl)butoxy)-2(1H)-quinolinone. The mechanism of action of CILO and several of its metabolites are cyclic AMP(cAMP) phosphodiesterase III inhibitors (PDE III inhibitors), inhibiting phosphodiesterase activity and suppressing cAMP degradation with a resultant increase in cAMP in platelets and blood vessels, leading to inhibition of platelet aggregation and vasodilation. (5)

Very few analytical methods have been reported for estimation of TELand CILO as a single ingredient as well as their combination with other drugs. The proposed method has been developed and validated for the determination of TEL and CILO. According to International Conference on Harmonization ICH Q2 (R1) guidelines, validation of the method was carried out by using accuracy, linearity, precision, the limit of detection, the limit of quantitation, system suitability, and specificity.

MATERIALS AND METHODS

Instruments used during the experiment were Electronic Weighing Balance of Sartorius - BT224S, Japan, UV Visible Spectrophotometer UV-1800, Pharmaspec, Shimadzu Ltd, Japan. FTIR Spectrophotometer WQF-520, USA. Along with that Methanol (Merck, India Limited), 0.1 N HCl, Analytical Reagent (Merck, India Limited) were also used.

Development of a simultaneous first-order method for the estimation of TEL and CILO

Preparation of stock solution of TEL

Accurately weighed 10 mg of TEL was transferred into 100 ml volumetric flask and dissolved in a small volume of methanol. The volume was adjusted to the mark with methanol to obtain the final concentration of TEL (100µg/ml). 1ml of this solution was transferred in 10 ml volumetric flask and volume was adjusted to the mark with gastric fluid (0.1 N HCl, to prepare a final concentration of 10µg/ml.

Preparation of stock solution of CILO

Accurately weighed 10 mg of CILO was transferred into 100 ml volumetric flask and dissolved in a small volume of methanol. The volume was adjusted to the mark with methanol to obtain a final concentration of CILO(100µg/ml). 1 ml of this solution was transferred in 10 ml volumetric flask and volume was adjusted to the mark with gastric fluid (0.1 N HCl, to prepare a final concentration of 10µg/ml.

Selection of analytical wavelength

Zero-order spectra of the standard solution of TEL and CILO was recorded and further, spectra were divided by 10 µg/mlTEL and 10µg/mlCILO respectively and these ratio spectra

of TEL and CILO were converted into first derivative and absorbance at wavelength 291.0 nm and 257.0 nm was determined for estimation TEL and CILO respectively.

Determination of TEL and CILO by first-order derivative Spectrophotometric method

A standard stock solution was used for the preparation of solution in the calibration range 2-14 µg /ml of TEL and 2-14 µg /ml of CILO in methanol in 10 ml of volumetric flask. The obtained ratio spectra of TEL were converted into the first-order derivative (D1) spectra. Likewise, ratio spectra of CILO were obtained by dividing the zero-order spectra of CILO (2 -14 µg/ml) by the spectrum of the standard solution of TELMI (10µg/ml). The obtained spectra of CILO were converted into the first-order derivative (D1) spectra with the interval of $\Delta\lambda$= 8 nm and scaling factor 20. The concentrations of selected analyte were quantified by measuring the amplitude maxima of respective analyte from their first-order derivative (D1) spectrum at a wavelength of 257.21 nm and 268.60 nm for TELMI and CILO respectively.

Validation of the proposed method

The developed method has been validated according to ICH guideline by determination of various analytical method validation parameters. The parameters studied are linearity, sensitivity, precision, and accuracy.

Linearity

The linearity was evaluated through linear regression analysis. The linearity for TELMI (2 – 14 µg/ml) at 257.21 nm and CILO (2-14 µg/ml) 268.60 nm was determined in terms of the correlation coefficient. The linearity was further confirmed by Bartlett's test, to evaluate the homoscedasticity of variance.

Limit of detection and limit of quantitation

The detection limit and quantitation limit was determined from calibration curves of TELMI and CILO. The limit of detection (LOD) was calculated using the formula, according to ICH guideline.

Precision

Precision was evaluated by performing repeatability and intermediate precision. The concentration of TEL and CILO selected within linearity range were 4µg/ml, 10µg/ml,

14µg/ml and 4µg/ml, 10µg/ml, 14µg/ml respectively. Three replicates for each concentration for TELMI and CILO were analyzed for precision. Overall mean and %RSD were calculated.

Accuracy

To demonstrate the accuracy of the proposed method, recovery studies were carried 3µg/ml for TELMI and 12µg/ml was spiked with 50%, 100% and 150% concentration of standard for TELMI (3,6, 12µg/ml) and for CILO (6,12,18µg/ml) respectively. %recovery was calculated by using the regression equation.

Calibration curve of first-order derivation of TEL and CILO

The aliquots of both drugs (TEL and CILO) were converted to first derivatives spectra and derivative absorbance at 257.21 and 268.60 nm for the estimation respectively.

RESULTS AND DISCUSSION

Development and Validation of UV First Order Derivative Spectrophotometric Method

Selection of analytical wavelength

Both the drugs TEL and CILO were showing good solubility in 0.1 N HCl and hence for the present analytical method, 0.1 N HCl was selected as the solvent. The zero-order spectra of TEL and CILO (10 µg/ml) each in the scanned range of 400-200 nm are as shown in Figure 5.1. The wavelength of maximum absorbance for TEL and CILO was found as 291 nm and 257 nm respectively. Zero-order spectra were transformed to first-order spectra using delta lambda 8 and scaling factor 20.

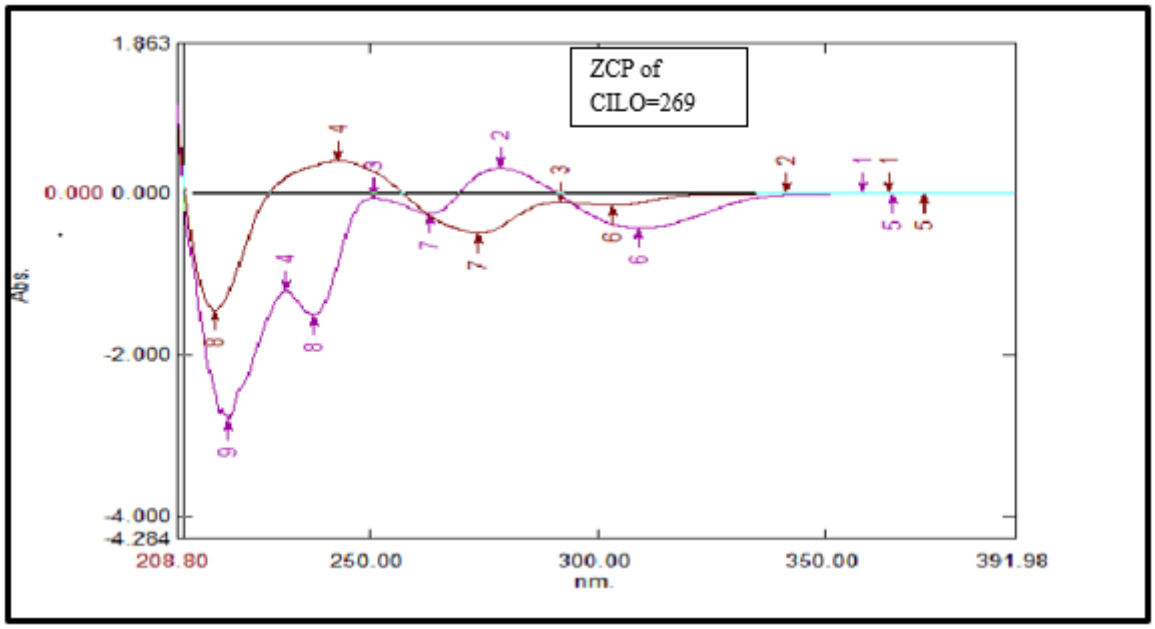

Figure No. 1: Overlay of First-order spectra of TEL (10 µg/ml) and CILO (10 µg/ml)

Calibration curve

Under the experimental conditions described, standard calibration curves for TEL and CILO were constructed by plotting absorbance versus concentration, respectively. Conformity for Beer's law was evident in the concentration range from 2- 14 µg/ml of each TEL and CILO (Figure 3). For quantitative analysis, the analytical data for the calibration graphs are shown in Table 5.1.

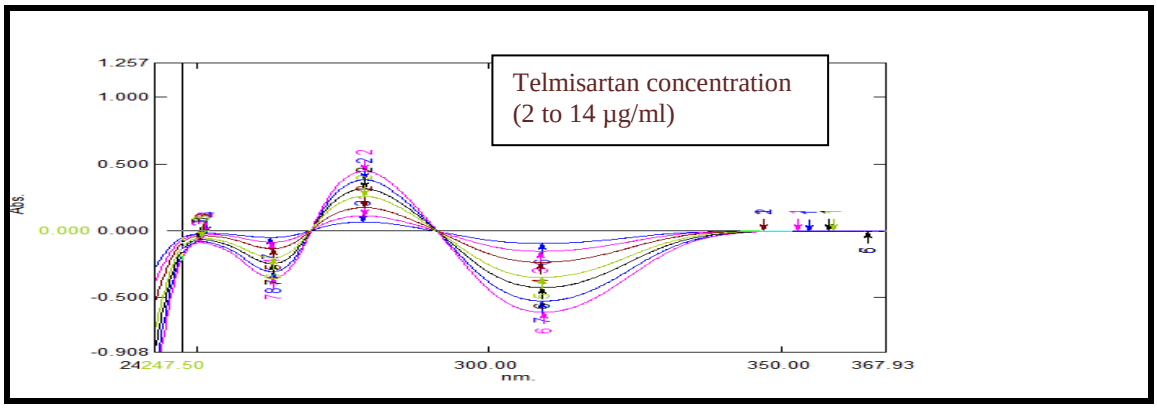

Figure No. 2: First order spectra of TEL (2-14µg/ml)

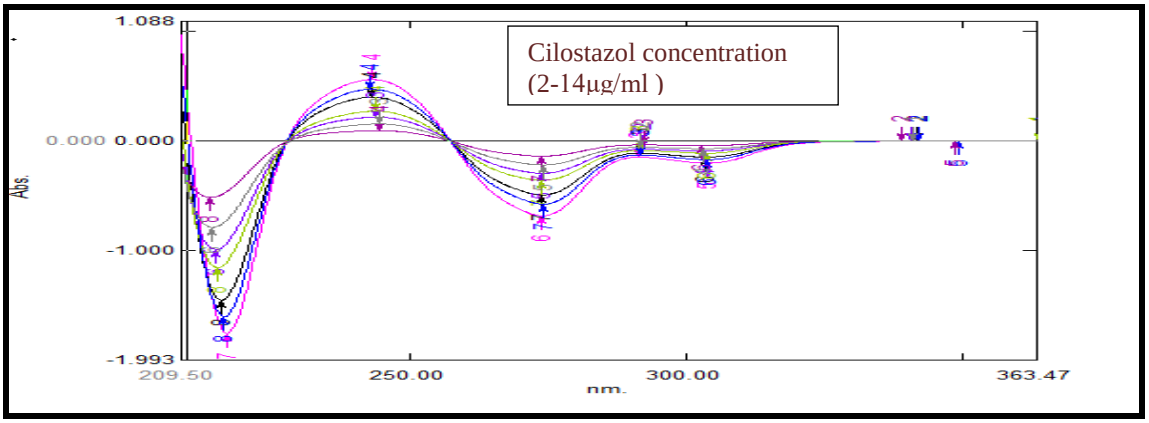

Figure No. 3: First-order spectra of CILO (2-14µg/ml)

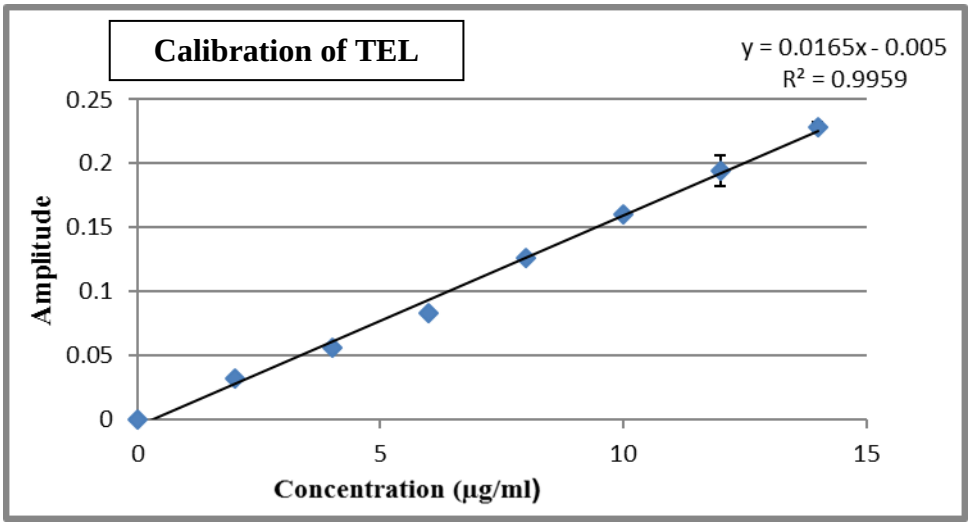

Figure No. 4: Calibration curve for TEL

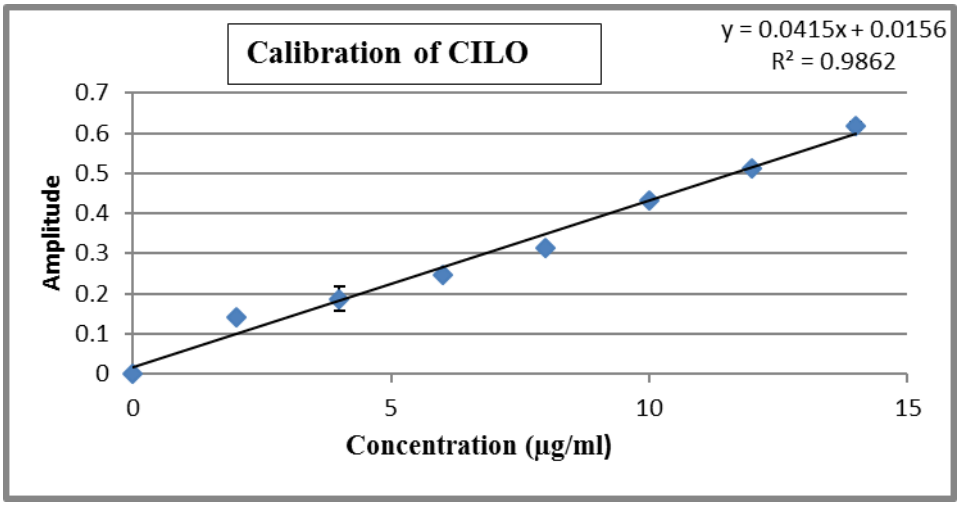

Figure No. 5: Calibration curve for CILO

Table No. 1: Linear regression parameters for TEL and CILO by first order derivative method

Parameters	TEL	CILO
Calibration range (µg/ml)	2-14	2-14
Regression equation[a]	y =0.0645x - 0.0171	y = 0.0642x + 0.0437
Standard deviation of slope	0.00007	0.00004
Confidence limit of slope[c]	0.0199-0.02004	0.0880-0.0881
Standard deviation of intercept	0.00029	0.0003
Confidence limit of intercept[c]	0.00102-0.00498	0.00670-0.00719
Correlation coefficient (r^2)	0.999	0.997
LOD (µg/ml)	0.041	0.01225
LOQ (µg/ml)	0.125	0.037121
Bartlett's test [b] (χ^2)	0.000110124	0.0000056

[a]mean of five replicates,[b] $\chi 2_{(0.05, 7)}$ value <14.067 at 95 % confidence interval for TEL and [b] $\chi^2_{(0.05, 7)}$ value <14.067 at 95 % confidence interval for CILO. Confidence interval at 95% confidence level and seven degree of freedom(t=1.895).

The linearity of the calibration curve and adherence of system to Beer's law was confirmed by a high value of correlation coefficient. Further homoscedasticity of variance for the response concerning the concentration range of 2-14 µg/ml for TEL and 2-14 µg/ml for CILO was also validated by Bartlett's test. The results showed that the calculated χ2 value is less than the critical value at 95% confidence interval, χ2 (0.05, 7) = 14.067 for TEL and χ2 (0.05, 7) = 14.067 for CILO; thus indicating that the variance of response is homogeneous.

Validation of the proposed analytical method

Precision

Three replicate determinations at different concentration levels were carried out to test the precision of the method. The relative standard deviation was found to be less than 2 %, indicating reasonable repeatability and reproducibility of the proposed method. The results of interday and intraday precision are as shown in Table 2.

Table No. 2: Precision study of the proposed method

Conc. µg/ml	Amplitude			% Recovery			SD	Mean % assay	% RSD
	1	2	3	1	2	3			
Repeatability for TEL									
4	0.093	0.092	0.093	95.74	94.68	95.74	0.61	95.39	0.643
10	0.232	0.234	0.232	97.44	98.29	97.44	0.49	97.73	0.502
16	0.378	0.375	0.377	99.73	98.93	99.46	0.4	99.37	0.408
Intermediate precision									
Day 1									
4	0.094	0.093	0.096	96.80	95.74	98.93	1.62	97.16	1.672
10	0.236	0.234	0.232	99.14	98.29	97.44	0.85	98.29	0.865
16	0.378	0.373	0.376	99.73	98.4	99.2	0.66	99.11	0.675
Day 2									
4	0.095	0.096	0.094	97.87	98.93	96.80	1.06	97.87	1.086
10	0.237	0.234	0.231	99.57	98.29	97.02	1.27	98.29	1.298
16	0.378	0.375	0.379	99.734	98.93	97.34	1.21	98.67	1.235
Repeatability for CILO									
4	0.334	0.332	0.334	96.73	96.16	97.30	0.56	96.73	0.586
10	0.866	0.867	0.865	99.08	99.19	98.96	0.11	99.08	0.114
16	1.369	1.367	1.368	97.60	97.46	97.53	0.07	97.53	0.072
Intermediate precision									
Day 1									
4	0.335	0.333	0.331	97.02	96.45	95.88	0.56	96.45	0.588
10	0.861	0.867	0.869	98.51	99.19	99.42	0.47	99.04	0.477
16	1.375	1.380	1.372	98.03	98.38	97.82	0.28	98.08	0.292
Day 2									
4	0.337	0.334	0.339	97.58	96.73	98.15	0.71	97.49	0.732
10	0.863	0.864	0.859	98.74	98.85	98.28	0.30	98.62	0.304
16	1.372	1.381	1.374	97.82	98.46	97.96	0.33	98.08	0.341

Accuracy

The proposed method offered the recovery of 97.58 to 99.57% for TEL and 97.17 to 99.89% for CILO after spiking the standard drug at 3 concentration levels of 50, 100 and 150 %. The values of % recovery and % RSD are as shown in Table 3 for both drugs. % RSD was found to be less than 2 % indicating that the developed method is accurate.

Table No. 3: Accuracy study for TEL and CILO by the proposed method

Recovery Level	Conc. of Sample (µg/ml)	Conc. of Std Spiked (µg/ml)	Total conc. taken (µg/ml)	Total conc. of the drug found (µg/ml)	% Recovery	Mean % recovery	SD	%RSD
For TEL								
50	2.5	5	7.5	7.31	97.58	98.53	0.86	0.879
	2.5	5	7.5	7.44	99.29			
	2.5	5	7.5	7.40	98.72			
100	5	5	10	9.95	99.57	99.14	0.42	0.429
	5	5	10	9.87	98.72			
	5	5	10	9.91	99.14			
150	7.5	5	12.5	12.38	99.06	99.06	0.34	0.343
	7.5	5	12.5	12.34	98.72			
	7.5	5	12.5	12.42	99.40			
For CILO								
50	2.5	5	7.5	7.28	97.17	97.63	0.45	0.465
	2.5	5	7.5	7.32	97.63			
	2.5	5	7.5	7.35	98.08			
100	5	5	10	9.92	99.21	98.64	0.51	0.527
	5	5	10	9.85	98.53			
	5	5	10	9.81	98.19			
150	7.5	5	12.5	12.48	99.89	99.10	0.73	0.740
	7.5	5	12.5	12.30	98.44			
	7.5	5	12.5	12.37	98.98			

[A] mean of three replicates at three concentration level

Analysis of marketed formulation

The developed method in the present study had used to quantify TEL and CILO. In the multiparticulate dosage forms (pellets TEL and CILO) were quantified using the proposed analytical method and the results are given in Table 4 indicates that the method can be routinely used for the analysis of novel formulation of pellets.

Table No. 4: Analysis of marketed formulation

Label claim		First-order derivative method	
		% Mean Recovery [a]	
TEL	CILO	TEL	CILO
10	50	98.67	97.64
% RSD		0.6793	0.6590

CONCLUSION

The first derivative spectrophotometric method was successfully applied in the dissolution study of formulated novel pellet formulation. On the other hand, the fundamental advantages of the investigated method are the simultaneous analysis of the mixture of both the drugs, without chemical pre-treatment, speed of analysis and cost-effectiveness. Hence, the developed method is simple, accurate, rapid, economical and precise and LOD and LOQ obtained are in range as per ICH guidelines. Finally, the developed methods can be applied to the routine analysis, quality control of mixtures and commercial preparations containing these drugs.

REFERENCES

1. Nair M. Keywords : Diabetes. 5thed.New Delhi; 2007.
2. V. M, S. Epidemiology of type 2 diabetes: Indian scenario. *Indian J Med Res*.2007;125:217–230.
3. Vikash K, Kumar MS, Amit L, Ranjit S. Multiple unit dosage form - pellet and pelletization techniques: An overview. *Int J Res Ayurveda Pharm*. 2011;2:121–125.
4. https://www.drugbank.ca/drugs/DB00966
5. https://www.drugbank.ca/drugs/DB01166