

RESEARCH ARTICLE

A NEW RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF TELMISARTAN AND CILOSTAZOL IN SYNTHETIC MIXTURE**Riddhi R. Patel and Dilip G. Maheshwari**

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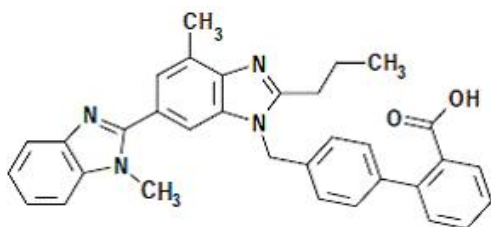
ABSTRACT

A simple, sensitive, precise and accurate RP-HPLC method has been developed for simultaneous estimation of Telmisartan and Cilostazol in the synthetic mixture by gradient elution. Chromatographic elution has been carried out on an Enable C18G (250 × 4.6 mm, 5 μm) column by using the mobile phase Potassium dihydrogen phosphate buffer (10mM): Methanol: Acetonitrile (30:10:60 v/v/v, pH 5.8). The flow rate was 1.0 ml/min. Detection was monitored at 257 nm using UV detector. The retention times of Cilostazol and Telmisartan was found to be 5.49±0.1min and 9.62±0.1 min respectively. The linearity was observed in the concentration range of 2-10 μg/ml for the Telmisartan and 4-20 μg/ml for the Cilostazol. The methods were validated and shown linear response for Telmisartan and Cilostazol. The limit of detection and limit of quantification for Telmisartan was found to be 0.041 and 0.125 μg/ml, respectively and for Cilostazol was found to be 0.038 and 0.117 μg/ml, respectively. The precision (intra-day, inter-day, repeatability) of methods were found within limits (RSD <2%). Accuracy was determined by recovery studies. Validation of proposed methods was carried out according to ICH guidelines.

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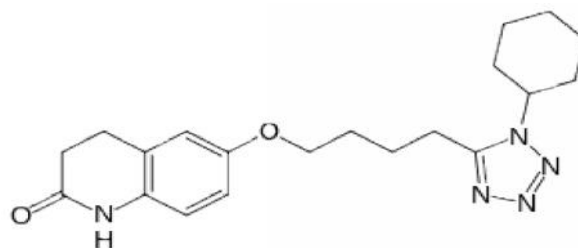
INTRODUCTION

Telmisartan is chemically 4'-[(1, 4'-dimethyl-2'-propyl [2, 6'-bi-1H-benzimidazol]-1'-yl) methyl]-[1, 1'-biphenyl]-2-carboxylic acid (Figure 1)^[1]. It is angiotensin II type 1 receptor antagonist, which selectively inhibit angiotensin II AT1 receptor and lowers the blood pressure in hypertensive patients. Telmisartan works by blocking the vasoconstrictor and aldosterone secretory effects of angiotensin II. Telmisartan is a partial agonist of PPAR (Nuclear peroxisome proliferator-activated receptor), which is an established target for the antidiabetic condition^[2-3]. Telmisartan is official in IP^[4], BP^[5] and USP^[6].

**Figure 1** Telmisartan

Literature review reveals that, various analytical methods have been reported for the estimation of Telmisartan in biological fluids, pharmaceutical formulation and bulk drug include UV spectrophotometric^[7-8], High-performance liquid chromatography method (HPLC)^[9], Stability indicating RP-HPLC method^[10-13], HPTLC method^[14], TLC-Desitometry

method^[15], LC/MS/MS method^[16] in individual and/or in combination of other drug.

**Figure 2** Cilostazol

Cilostazol is chemically 6- [4- (1- cyclohexyl- 1H- tetrazol- 5-yl) butoxy]- 3, 4- dihydro- 2 (1H)- quinolinone (Figure 2)^[17]. It is cyclic adenosine monophosphate phosphodiesterase III inhibitors, inhibiting phosphodiesterase activity and suppressing cAMP degradation with a resultant increase in cAMP in platelets and blood vessel leading to inhibition of platelet aggregation and vasodilation^[2]. Therefore, Cilostazol is used for the treatment of intermittent claudication resulting from peripheral arterial diseases. Cilostazol is official in IP^[4] and USP^[6]. Based on literature survey, it was found that Cilostazol have been analysed by Spectrophotometry^[18], HPLC in pharmaceutical formulation alone and in combination with other drugs^[19]. Both the drug in combination gives desirable synergistic effect on hyperglycaemia and hypertriglyceridemia^[20]. Literature review shows that, there is no reported method available for simultaneous estimation of both the drugs in combination. Therefore it is thought of interest to developed

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simple, accurate, precise and validated RP-HPLC method for simultaneous estimation of Telmisartan and Cilostazol in combination.

MATERIALS AND METHODS

Instrumentation

The chromatographic separation was carried out using LC-2010 CHT equipped with UV detector. Lab solution software was used for monitor the output single at wavelength 257 nm. Drug separation was achieved at room temperature with Enable-C18G (4.6mm ×250mm, 5µm) column. Electronic analytical balance (Wensar DA103) was used for weighing.

Chemicals and Reagents

The bulk drug Telmisartan was obtained from (Virdev Intermediates Pvt., Surat, Gujarat) and Cilostazol was obtained from (Cadila Pharma Ltd., Ahmedabad, Gujarat. All used solvents were of HPLC grade. Methanol, ACN, Ortho phosphoric acid, obtained from Merck Pvt. Ltd.

Preparation of Buffer (10 mM K₂HPO₄)

Accurately weighed quantity of 1.36 gm Potassium dihydrogen phosphate (K₂HPO₄) was transferred in 1000 ml beaker, dissolved in 200 ml HPLC grade water and sonicated for about 20 min and diluted up to the mark with HPLC grade water. It was filtered through 0.45 µm membrane filter. Buffer pH was adjusted to 3.65 using 1% ortho phosphoric acid.

Preparation of Standard solutions and synthetic mixture

A. Preparation of standard stock solution of Telmisartan (100 µg/ml)

Accurately weigh 10 mg of Telmisartan was transferred into a 100 ml volumetric flask and dilute upto mark with Methanol.

B. Preparation of standard stock solution of Cilostazol (100 µg/ml)

Accurately weigh 10 mg of Cilostazol was transferred into a 100 ml volumetric flask and dilute upto mark with Methanol.

C. Preparation of synthetic mixture of Telmisartan and Cilostazol

The synthetic mixture of Telmisartan and Cilostazol was prepared in the ratio of 1:4. Accurately weighed Telmisartan (10 mg) and Cilostazol (40 mg) were transferred in 100 ml volumetric flask and dissolved in Methanol. Common excipients, Lactose, Magnesium Stearate, Meglumine, HPMC etc which are used in the tablet formulation, were added in this mixture and sonicated for 20 minutes. This solution was filtered through Whatman filter paper. The filtrate was diluted to the mark with Methanol. The mixture contains 100 µg/ml of Telmisartan and 400 µg/ml of Cilostazol.

Preparation of sample solution

From the synthetic mixture solution take 0.5 ml and transferred in to a 10 ml volumetric flask and the volume was adjusted up

to the mark with mobile phase to make final concentration of Telmisartan 5 µg/ml and Cilostazol 20 µg/ml.

Selection of wavelength for estimation of Telmisartan and Cilostazol

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. At 257 nm both the drug give good peak height and shape. So, 257 nm was selected for simultaneous estimation of Cilostazol and Telmisartan.

Calibration curve for Telmisartan and Cilostazol

For Telmisartan

An aliquots of stock solution of Telmisartan 0.2, 0.4, 0.6, 0.8 and 1.0 ml were pipette out in five different 10 ml volumetric flasks and further diluted with mobile phase to obtain the concentration of about 2, 4, 6, 8 and 10 µg/ml respectively. Graph of Area Vs Concentration was plotted.

For Cilostazol

An aliquots of stock solution of Cilostazol 0.4, 0.8, 1.2, 1.6 and 2.0 ml were pipette out in five different 10 ml volumetric flasks and further diluted with mobile phase to attain concentration of about 4, 8, 12, 16 and 20 µg/ml respectively. Graph of Area Vs Concentration was plotted.

Optimization of Chromatographic conditions

Various mobile phases, such as Methanol: Water, Acetonitrile: Water, Phosphate Buffer: Acetonitrile, Phosphate Buffer: Methanol in different proportions was tried. The combination of Pottassium dihydrogen phosphate (pH 3.65, adjusted with 1% ortho phosphoric acid): Methanol: ACN (30:10:60 v/v/v) provide the optimum polarity for proper migration, separation and resolution of Cilostazol and Telmisartan. Under these conditions, the eluted peaks were well defined and properly resolved. The retention time of Cilostazol and Telmisartan was found to be 5.49 min and 9.62 min, respectively at a flow rate of 1.0 ml/min.

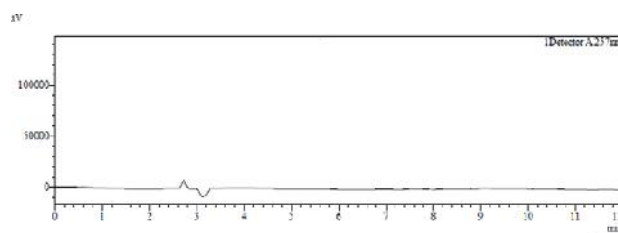


Figure 3 Blank chromatogram (Solvent peak)

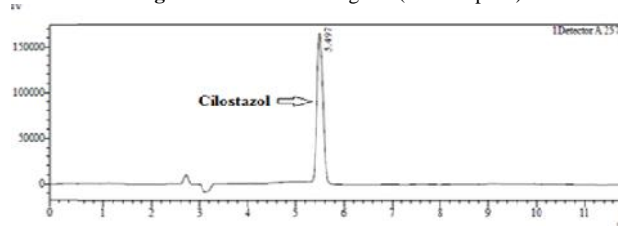


Figure 4 HPLC chromatogram of Cilostazol (20 µg/ml)

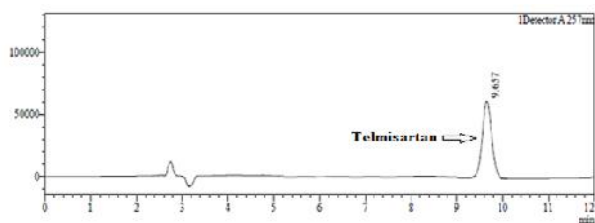


Figure 5 HPLC chromatogram of Telmisartan (10 µg/ml)

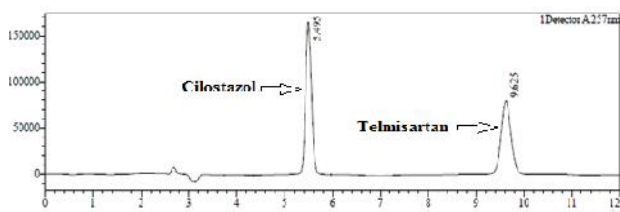


Figure 6 HPLC chromatogram of Cilostazol (20 µg/ml) and Telmisartan (10 µg/ml)

calibration curve of Area (mv) vs Concentration (µg/ml) and measure the correlation coefficient and regression equations for Telmisartan and Cilostazol.

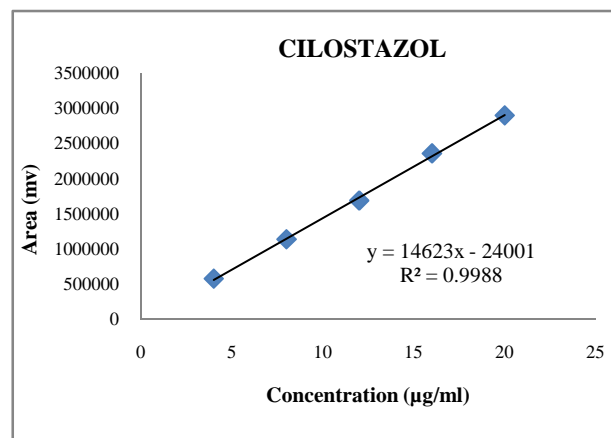


Figure 8 Calibration curve of Cilostazol (4-20 µg/ml)

System suitability parameters

The resolution, tailing factor and number of theoretical plates are shown in Table. The values obtain confirmed the suitability of the system for the analysis of these drugs in combination.

Table 1 System suitability parameters for Telmisartan and Cilostazol

Sr. No	System suitability parameter	CILO Mean ± S.D.(n=3)	TEL Mean ± S.D. ss(n=3)
1	Retention time	5.495	9.625
2	Theoretical Plates	8047	9375
3	Tailing Factors	1.099	1.027
4	Resolution	-	12.850

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Intraday Precision

Solutions containing 4, 6, 8 µg/ml of TEL and 8, 12, 16 µg/ml of CILO were analyzed three times on the same day and %R.S.D was calculated.

Table 2 Calibration data for Telmisartan (2-10 µg/ml) and Cilostazol (4-20 µg/ml)

Sr. No	Concentration (µg/ml)		Mean Peak area (µv*sec)±S. D. (n=5)		% RSD	
	TEL	CILO	TEL	CILO	TEL	CILO
1	2	4	498783.3 ± 2088.28	577422.2 ± 2553.11	0.4186	0.4421
2	4	8	717120.7 ± 2520.21	1141288 ± 6418.28	0.3514	0.5623
3	6	12	906160.3 ± 4224.56	1678590 ± 8246.41	0.4662	0.4912
4	8	16	1083380 ± 6185.81	2361872 ± 9696.62	0.5709	0.4105
5	10	20	1321168 ± 8472.35	2881635 ± 19267.69	0.6412	0.6686

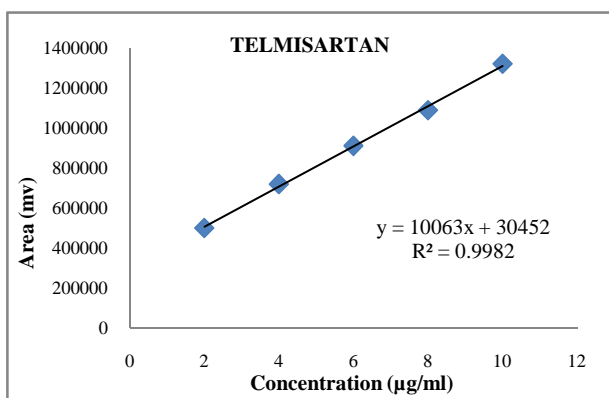


Figure 7 Calibration curve of Telmisartan (2-10 µg/ml)

Table 3 Intraday Precision study for Telmisartan and Cilostazol

Drug	Concentration(µg/ml)	Mean peak area (µv*sec) ± S.D (n=3)	% RSD
Telmisartan	4	719554.2 ± 4326.52	0.6012
	6	907406 ± 5350.64	0.5896
	8	1085053 ± 8074.34	0.7441
Cilostazol	8	1148112 ± 7533.60	0.6561
	12	1684485 ± 13294.12	0.7892
	16	2379394 ± 15867.66	0.6668

Table 4 Interday Precision study for Telmisartan and Cilostazol

Drug	Concentration(µg/ml)	Mean peak area (µv*sec) ± S.D (n=3)	% RSD
Telmisartan	4	721169.6 ± 6669.66	0.9248
	6	912467.6 ± 9685.96	1.0615
	8	1079760 ± 9120.64	0.8446
Cilostazol	8	1149850 ± 13761.87	1.1968
	12	1682128 ± 16446.73	0.9777
	16	2359598 ± 21072.18	0.8930

Method Validation [21]

Linearity & Range

The linearity of Telmisartan and Cilostazol was found to be in the range of 2-10 µg/ml and 4-20 µg/ml respectively. Plot the

Interday Precision

Solutions containing 4, 6, 8 µg/ml of TEL and 8, 12, 16 µg/ml of CILO were analyzed on three different successive days and %R.S.D was calculated.

Repeatability

Solutions containing 6 µg/ml of TEL and 12 µg/ml of CILO were analyzed for six times and % R.S.D. was calculated. % R.S.D was not more than 2%.

Table 5 Repeatability study for Telmisartan and Cilostazol

Drug	Concentration(µg/ml)	Mean peak area (µv*sec) ± S.D (n=6)	% RSD
Telmisartan	6	906139.8 ± 2904.10	0.3204
Cilostazol	12	1682936 ± 7878.83	0.4681

$$LOQ = 10 \times (/ S)$$

Where, = standard deviation of the Y intercept of calibration curve

S = Mean slope of the corresponding calibration curve.

LOD values for TEL and CILO were found to be 0.041 µg/ml and 0.038 µg/ml, respectively. LOQ values for TEL and CILO were found to be 0.125 µg/ml and 0.117 µg/ml respectively.

Accuracy

Accuracy of the developed method was confirmed by doing recovery study as per ICH guideline at three different concentration levels 80%, 100%, 120% and the values were measured at all wavelengths for Telmisartan and Cilostazol.

Table 6 Accuracy study data

Name of Drug	% Level of recovery	Amount of drug Taken (µg/ml)	Amount of drug added (µg/ml)	Total amount Taken (µg/ml)	Total amount found (µg/ml)	% Recovery ± S.D.(n=3)
Telmisartan	80	2	1.6	3.6	3.63	100.16 ± 0.5852
	100	2	2	4	3.94	98.31 ± 0.7447
	120	2	2.4	4.4	4.37	99.34 ± 0.3863
Cilostazol	80	8	6.4	14.4	14.32	99.66 ± 0.6033
	100	8	8	16	16.11	100.95 ± 0.6850
	120	8	9.6	17.6	17.55	98.16 ± 0.7238

Table 7 Application of HPLC method to synthetic mixture

Drug Name	Amount in synthetic mixture (mg)	Test conc. Taken (µg/ml)	Amount found (µg/ml)	Amount found (mg) ± SD (n=3)	% Recovery ±SD (n=3)
Telmisartan	10	5	5.04	10.08 ± 0.059	100.80 ± 0.6208
Cilostazol	40	20	19.93	39.86 ± 0.074	99.65 ± 0.4099

Table 8 Summary of validation parameters

Sr. No.	Parameters	Cilostazol	Telmisartan
1	Beer's Law Limit (µg/ml)	4-20	2-10
2	Regression equation (y = mx +c)	y = 14623x-24001	y = 10063x+30452
3	Correlation Coefficient (r ²)	0.998	0.998
4	Repeatability (% RSD, n=6)	0.46	0.32
5	Intraday Precision (% RSD, n=3)	0.65-0.78	0.58-0.74
6	Interday Precision(% RSD, n=3)	0.84-1.06	0.89-1.19
7	LOD (µg/ml)	0.038	0.041
8	LOQ (µg/ml)	0.117	0.125

LOD and LOQ

Limit of Detection (LOD)

Limit of detection can be calculated using following equation as per ICH guidelines.

$$LOD = 3.3 \times (/ S)$$

Where, = standard deviation of the Y intercept of calibration curve

S = Mean slope of the corresponding calibration curve.

Limit of Quantification (LOQ)

Limit of quantification can be calculated using following equation as per ICH guidelines.

This performance was done in triplicate. From the recovery study it was clear that the method is very accurate for quantitative estimation of Telmisartan and Cilostazol in mixture and the results were within the acceptance range.

Analysis of synthetic mixture

Applicability of the proposed method was tested by analyzing the synthetic mixture.

RESULT

DISCUSSION

A simple, sensitive, accurate, precise, reverse phase high performance liquid chromatography (RP-HPLC) method has been developed which can be used for quantitative estimation

of Telmisartan and Cilostazol for routine analysis of individual and combination of drugs. Method was validated as per ICH Q2 (R2).

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