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## METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUS ESTIMATION OF CILOSTAZOL AND TELMISARTAN

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### Research Article

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#### ABSTRACT

Three rapid, sensitive and specific UV-Visible methods were developed and validated for the estimation of Cilostazol and Telmisartan in synthetic mixture. All method was validated according to ICH guideline in terms of linearity, accuracy, precision, and specificity, limit of detection and limit of quantification. The first method is simultaneous equation method. Method I is based on simultaneous equation. Cilostazol and Telmisartan show absorbance maximum at 258 and 296 nm respectively, so absorbance was measured at the same wavelength for estimation of Cilostazol and Telmisartan. Method II is based on determination of Q-value. Absorbance is measured at 237.5 nm (Iso absorptive point) and 258 nm ( $\lambda$  max of Cilostazol). Method III is dual wavelength method, in which two were selected for each drug in a way so that the difference in absorbance is zero for another drug. Both drugs obey the Beer Lambert's law in the concentration range of 1-40  $\mu$ g/ml for Cilostazol and 1-25  $\mu$ g/ml for Telmisartan.

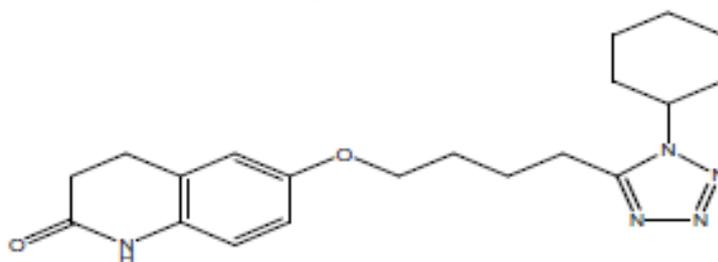
### INTRODUCTION

Simultaneous estimation of drug is very important as the new combined formulation approved in market. Simultaneous estimation of drug allows the estimation of drug without prior separation of drug. So, it saves the time of analysis and also the cost of analysis because all the material and reagent require for analysis are common for the entire drug in combined formulation. Simultaneous estimation of drug is more economical for estimation of combined drug formulation, and it is widely used for the quantification of drug in combination.

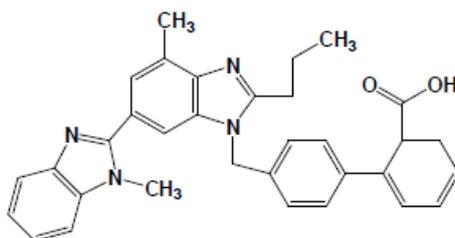
Cilostazol, whose chemical name is 6-[4-(1-cyclohexyl-1H-tetrazol-5-yl) butoxy]-3, 4-dihydro-2 (1H)-quinolinone (**Figure 1**), is a quinolinone derivative that inhibits cellular phosphodiesterase III, and is used for the inhibition of platelet aggregation and as a vasodilator [1,2]. Telmisartan is 4'-([4-methyl-6-(1-methyl-1H-benzimidazol-2-yl)-2-propyl-1H-benzimidazol-1-yl]methyl)-2- biphenyl carboxylic acid (**Figure 2**). Telmisartan is a new angiotensin II receptor antagonist for the treatment of essential hypertension with a lower incidence of cough than ACE inhibitors [3,4].

Several methods for determination of Cilostazol and Telmisartan individual and in combination with other drug have been reported in the past, such as spectrophotometric [5-9]. A new RP-HPLC method for simultaneous estimation of Cilostazol and Telmisartan also has been developed. Although RP-HPLC method for simultaneous estimation of Cilostazol and Telmisartan and spectrophotometric method for estimation of Cilostazol and Telmisartan individual and in combination with other drug method has

been developed to quantify Cilostazol and Telmisartan but no Spectrophotometric method has been developed for simultaneous estimation of Cilostazol and Telmisartan in combined dosage form<sup>[10-15]</sup>.



**Figure 1.** Chemical structure of Cilostazol.



**Figure 2.** Chemical structure of Telmisartan.

Present paper represents three spectrophotometric method for the simultaneous estimation Cilostazol and Telmisartan in synthetic mixture.

A simple, rapid, accurate, precise, reliable and economical RP- HPLC method with UV detection was optimized, developed and validated as per ICH-Q2 guideline for the simultaneous estimation of Cilostazol (CLZ) and Telmisartan (TLM) in tablet dosage form. The developed method was validated in terms of accuracy, precision, and linearity, limit of detection and limit of quantification. From the validation outcomes it was established that proposed method can be used for the approximation of both drugs in combined pharmaceutical tablet dosage form.

## MATERIALS AND METHODS

### Instruments

A Shimadzu model 1700 double beam UV-Visible spectrophotometer with spectral width of 1 nm, wavelength accuracy of  $\pm 0.1$  nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software (Ver.2.1). The samples were weighed on electronic analytical balance (A $\times$ 120, shimadzu).

### Materials

All chemicals and reagents were used of AR grade (Thermo Fisher scientific IND PVT. LTD Mumbai). Telmisartan was kindly gifted from Alembic pharmaceutical, Vadodara and Cilostazol was purchased from Swapnroop drug Pvt. Ltd Bombay.

### Selection of common solvent

After checking the solubility of drugs in water, acetonitrile and methanol solvents methanol has been selected as common solvent for developing spectral characteristics.

### Selection of detection wavelength

Solution was scanned over the range of 200-400 nm. It was observed that the drugs showed maximum absorbance at 258 nm for Cilostazol and 296 nm for Telmisartan were selected as the wavelength for detection.

### Preparation of standard stock solution

The standard stock solutions of Cilostazol and Telmisartan were prepared by dissolving 25 mg of each drug in methanol and final volume was adjusted with same solvent in 25 mL of volumetric flask to get a solution containing 1000  $\mu$ g/mL of each drug.

### Preparation of Working Standard Solution

From the above solution, further dilute 10 ml of stock solution up to 100 ml in volumetric flask to get second stock of 100  $\mu$ g/mL.

### Preparation of Calibration Curve of Standard

From working std. solution of CLZ (100  $\mu$ g/ml) 0.4, 0.8, 1.2, 1.6 and 2.0ml were transferred to 10 ml volumetric flasks and volume were made up to the mark with methanol. This gives 4 to 20  $\mu$ g/ml of CLZ. From working std. solution of TLM (100  $\mu$ g/ml)

0.1, 0.2, 0.3, 0.4 and 0.5ml were transferred to 10 ml volumetric flasks and volume were made up to the mark with methanol. This gives 1 to 5 µg/ml of TLM.

### Method I (Simultaneous equation method) [3,10,16,17]

If a sample contains two absorbing drug each of which absorbs at the λ max of the other, it may be possible to determine both drugs by the technique of simultaneous equation. Two wavelengths selected for the development of the simultaneous equations are 258 nm and 296 nm (Figure 3). The absorptivity values determined for Cilostazol at 258 and 296 are (ax1), and (ax2), and for Telmisartan are (ay1), and (ay2) at 258 nm and 296 nm respectively. These values are means of six estimations. The absorbance and absorptivity at these wavelengths were substituted in equation 1 and 2 to obtain the concentration of both drugs. Absorptivity values for both the drug are mentioned in Table 1 for calculation.

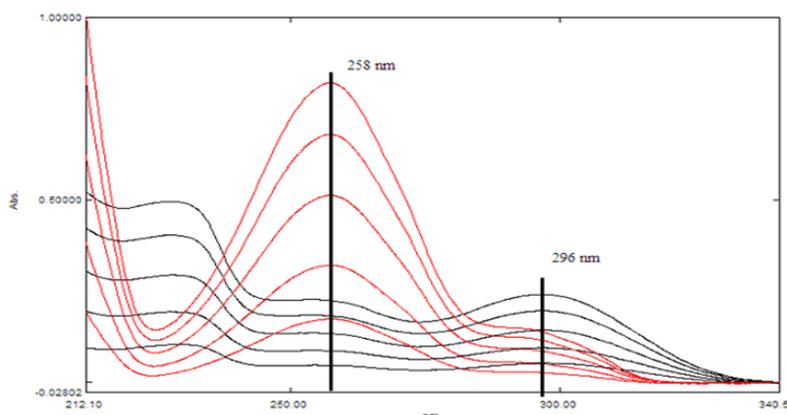


Figure 3. Zero ordered overlay spectra for CLZ (4-20 µg/ml Red) and TLM (1-5 µg/ml Black) (simultaneous method).

Table 1. Absorptivity values of CLZ and TLM for method I.

Sr No.	Absorptivity values for CLZ		Absorptivity values for TLM	
	At 258 nm (ax1)	At 296 nm (ax2)	At 258 nm (ay1)	At 296 nm (ay2)
1	402.525	72.625	543.200	561.500
2	414.887	69.275	469.350	506.600
3	412.600	71.916	474.033	509.866
4	411.225	73.168	474.250	520.325
5	414.065	72.085	478.280	523.200
Average	411.225	71.814	487.822	524.298

$$C_x (\text{CLZ}) = (A_2 a_{y1} - A_1 a_{y2}) / (a_{x2} a_{y1} - a_{x1} a_{y2}) \quad (1)$$

$$C_y (\text{TLM}) = (A_1 a_{x2} - A_2 a_{x1}) / (a_{x2} a_{y1} - a_{x1} a_{y2}) \quad (2)$$

Where,

A1 and A2=Absorbance of sample at 258 and 296,

Cx and Cy=Concentrations of Cilostazol and Telmisartan in sample matrix,

ax1 and ax2=Absorptivity's of Cilostazol at 258 and 296,

ay1 and ay2=Absorptivity's of Telmisartan at 258 and 296.

### Method II (Absorbance ratio method) [3,10,18,19]

Absorbance ratio method of analysis is based on the absorbance at two selected wavelengths, one of which is an Iso absorptive point and the other being the wavelength of maximum absorption of one drug. From overlain spectra of cilostazol and telmisartan (Iso absorptive point) (237.5) are selected for the formation of Q absorbance equation (Figures 4 and 5). The absorptivity values determined for cilostazol at 237.5 nm are (ax1), and 258 nm are (ax2) and for telmisartan at 237.5 nm are (ay1), at 258 nm are (ay2). These values are means of five estimations. The absorbance and absorptivity at these wavelengths were substituted in equation 3 and 4 to obtain the concentration of drugs. Absorptivity values for both the drug are mentioned in Table 2 for calculation.

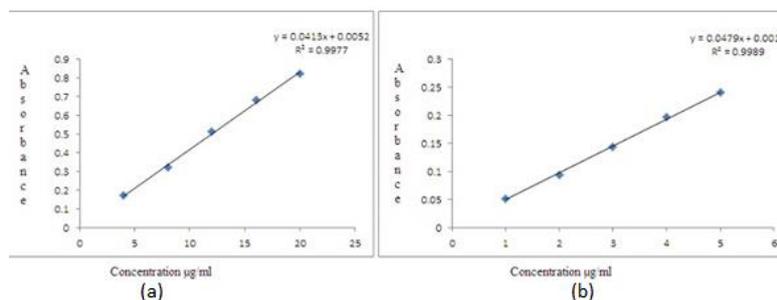
$$C_X = [(Q_M - Q_Y) / (Q_X - Q_Y)] \times A_1 / a_{x1} \quad (3)$$

$$C_Y = [(Q_M - Q_X) / (Q_Y - Q_X)] \times A_1 / a_{y1} \quad (4)$$

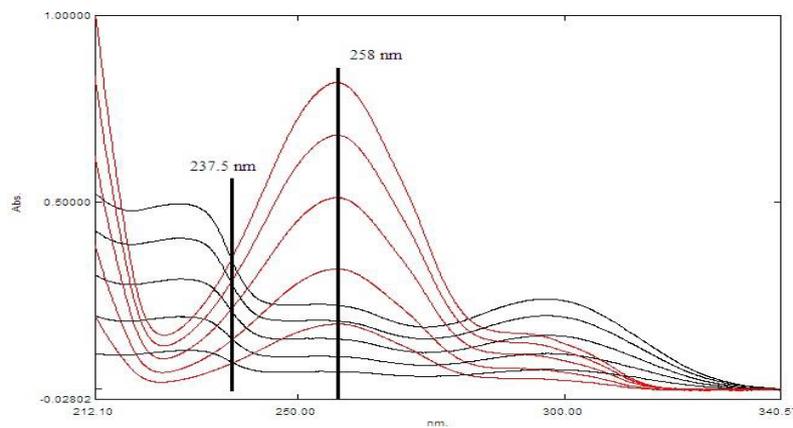
Where,

A1 and A2 are absorbance of mixture at 237.5 and 258 nm respectively,

ax1 and ay1 are absorptivity of cilostazol and telmisartan at 237.5nm respectively,  
 ax2 and ay2 are absorptivity of cilostazol and telmisartan at 258 nm respectively,  
 $QM=A2/A1$ ,  $QX=ax2/ax1$  and  $QY=ay2/ay1$ .



**Figure 4.** Calibration curve for (a) CLZ 4-20 µg/ml and (b) TLM 1-5 µg/ml for (simultaneous method).



**Figure 5.** Zero ordered overlay spectra for CLZ (4-20 µg/ml RED) and TLM (1-5 µg/ml) BLACK (Q-method).

**Table 2.** Absorptivity values of CLZ and TLM for method II.

Sr No.	Absorptivity values for CLZ		Absorptivity values for TLM	
	At 258 nm (ax1)	At 237.5 nm (ax2)	At 258 nm (ay1)	At 237.5 nm (ay2)
1	402.525	178.225	543.200	827.600
2	414.887	177.612	469.350	749.500
3	412.600	178.125	474.033	757.633
4	411.225	178.606	474.250	763.850
5	414.065	178.530	478.280	766.360
Average	411.225	178.219	487.822	772.988

### Method III Dual wavelength method [20-24]

Dual wavelength method is applicable when both the drug absorb at each other wavelength. Two wavelength points should select in such a way that difference between these two points is zero for one drug and at other drug should have significant absorbance. So, that both drug can be quantified at each other wavelength. From the, overlain spectra, four wavelengths 284 nm, 264.9 nm, 243.4 nm and 270.6 nm were selected (**Figures 6 and 7**) for quantitation of both the drugs by dual wavelength spectrophotometric method. The quantitative determination of Cilostazol is carried out by measuring the absorbance difference value at between 284 nm and 264.9 nm where Telmisartan has same absorbance at both the wavelength. The difference between 243.4 nm and 270.6 nm where Cilostazol has same absorbance at both wavelengths. This difference between these wavelengths is directly proportional to concentration.

## VALIDATION OF THE DEVELOPED METHODS

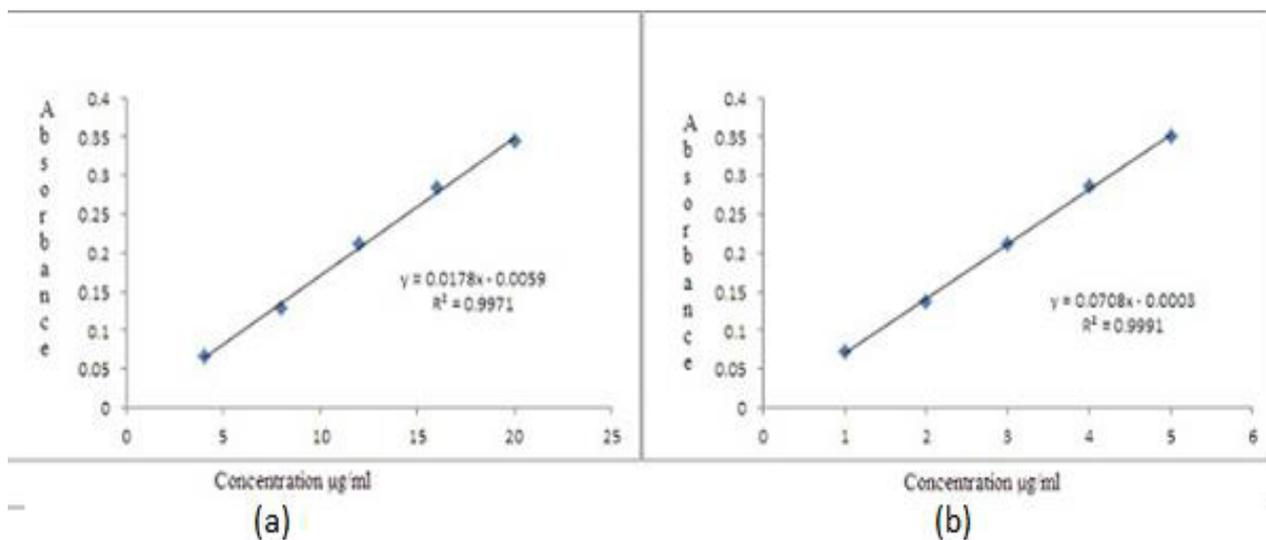
### Linearity

The linearity of all three proposed methods was evaluated by analyzing a series of five different concentrations of CLZ (4 to 20 µg ml<sup>-1</sup>) and TLM (1 to 5 µg ml<sup>-1</sup>) and each was repeated three times. The calibration graphs obtained under the experimental conditions described showed good linear relationships.

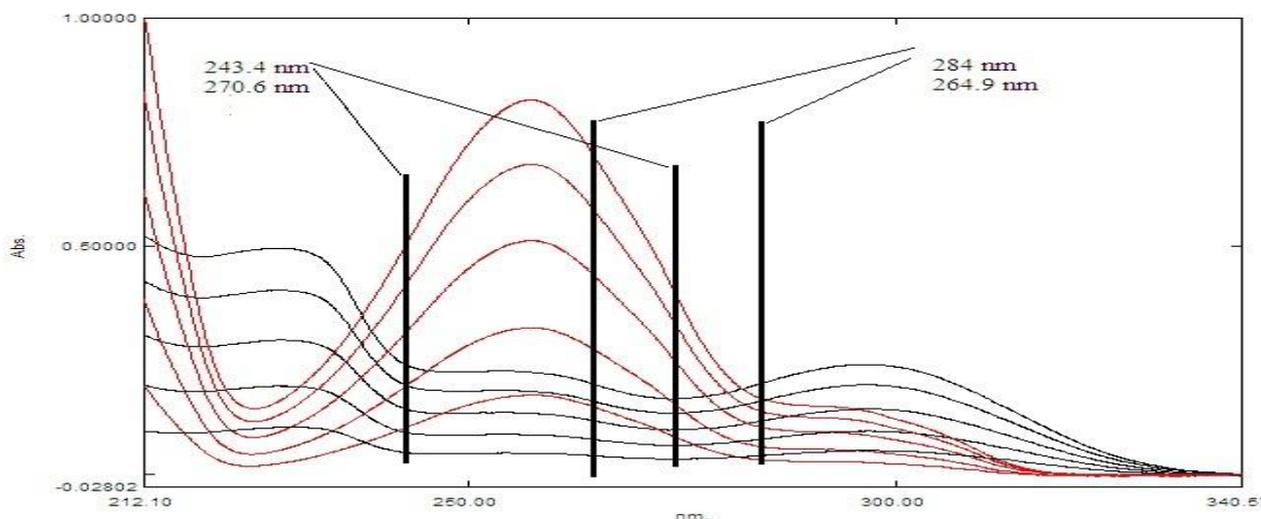
### Accuracy

To check the accuracy of the proposed method, recovery studies were carried out 80, 100 and 120% of the test concentration

as per ICH guidelines. The recovery study was performed three times at each level. The result of the recovery studies are reported in **Table 3**.



**Figure 6.** Calibration curve for (a) CLZ 4-20 µg/ml (b) TLM 1-5 µg/ml for (Q-method).



**Figure 7.** Zero ordered overlay spectra for CLZ (4-20 µg/ml Red) and TLM (1-5 µg/ml Black) (dual wavelength method). for (a) CLZ 4-20 µg/ml (b) TLM 1-5 µg/ml for (Q-method).

**Table 3.** Results of recovery study of CLZ and TLM by developed method.

Method	% spiking	C Actual µg/ml		C Added µg/ml		C Recover* µg/ml		% Recovery ± SD	
		CLZ	TLM	CLZ	TLM	CLZ	TLM	CLZ	TLM
I	80	16	4	12.8	3.2	13.16	3.18	102.81 ± 0.0608	99.375 ± 0.0000
	100	16	4	16	4	16.56	4.18	102.50 ± 0.0503	102.97 ± 0.0115
	120	16	4	19.2	4.8	20.05	4.88	101.44 ± 0.0650	101.66 ± 0.0346
II	80	16	4	12.8	3.2	13.16	3.24	102.81 ± 0.0005	101.25 ± 0.0115
	100	16	4	16	4	16.72	4.16	100.50 ± 0.0493	102.00 ± 0.0264
	120	16	4	19.2	4.8	20.13	4.86	99.864 ± 0.0600	101.25 ± 0.0404
III	80	16	4	12.8	3.2	12.87	3.07	100.54 ± 0.0461	97.812 ± 0.0896
	100	16	4	16	4	16.13	4.00	100.81 ± 0.0723	100.15 ± 0.0680
	120	16	4	19.2	4.8	19.61	4.84	102.13 ± 0.1274	100.83 ± 0.0888

### Precision

Inter-day and intra-day precision was measured in terms of % RSD. The experiment was repeated 3 times in a day for intra-day and on 3 different days for inter-day precision. The average % RSD of intra-day and inter-day measurements for determination of both the drugs was found to be less than 2 for all five methods (**Table 4**).

### Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by Calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

**Table 4.** Summary of validation parameter by developed method.

Parameter	Method I		Method II		Method III	
	CLZ	TLM	CLZ	Isoabsorptive point	CLZ	TLM
Analytical wavelength (nm)	258	296	258	237.5	284,264.9	243.4,270.6
Beer's range (µg/ml)	4.0-20.0	1.0-5.0	4.0-20.0	1.0-5.0	4.0-20.0	1.0-5.0
Slope	0.0413	0.0479	0.0178	0.0708	0.0262	0.0134
Intercept	0.0052	0.0015	-0.0059	-0.0003	0.0085	-0.0022
Correlation coefficient	0.9977	0.9989	0.9971	0.9991	0.9982	0.9982
Intraday precision (%RSD)	1.0442	0.5711	1.62806	0.98455	1.258	1.2397
Interday precision (%RSD)	0.5770	0.7287	1.1689	1.2821	0.8111	1.8552
LOD(µg/ml)	0.3355	0.0085	0.9181	0.12972	0.3668	0.1297
LOQ(µg/ml)	1.0166	0.2587	2.7823	0.6555	1.1115	0.3931

$$\text{LOD} = 3.3 \times \sigma/S$$

$$\text{LOQ} = 10 \times \sigma/S$$

Where,

$\sigma$ =the standard deviation of the response and

S=slope of the calibration curve.

## Robustness

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters. If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. In this method for robustness study different brand of methanol selected. The results of robustness study are shown in **Table 5**.

**Table 5.** Result of robustness study.

Factor	% Found					
	Method I		Method II		Method III	
	CLZ	TLM	CLZ	TLM	CLZ	TLM
Solvent						
Brand 1	102.50	102.97	100.50	102.00	100.81	100.15
Brand 2	102.37	101.51	102.28	99.437	99.983	101.53
Mean $\pm$ SD	101.93 $\pm$ 0.04	102.48 $\pm$ 0.03	101.39 $\pm$ 0.04	100.71 $\pm$ 0.03	100.39 $\pm$ 0.06	100.84 $\pm$ 0.05

## APPLICABILITY OF THE DEVELOPED UV SPECTROPHOTOMETRIC METHODS

All methods were successfully applied for the estimation of Cilostazol and Telmisartan in synthetic mixture.

### Preparation of synthetic mixture

Synthetic mixture was prepared using various the excipients in the pharmaceutical oral synthetic mixture (CLZ 40 mg and TLM 10 mg). Inactive ingredients of the formulation include MCC, copovidone, SSG, Mg stearate, cornstarch and Talc.

### Analysis of synthetic mixture

From synthetic mixture an amount equivalent to 4 mg CLZ and 16 mg TLM was weighed and dissolved in 100 ml methanol. Solutions were filtered using whatman filter paper grade 1. Appropriate dilutions were prepared in methanol using aliquots of the clear filtrates and subjected to analysis using all the three methods described above **Table 6**.

**Table 6.** Results of simultaneous estimation of synthetic mixture for method I, II, and III.

Formulation : synthetic mixture		
Drug	Composition of mixture	% Drug found* $\pm$ SD
Cilostazol	40 mg	102.475 $\pm$ 0.1150
Telmisartan	10 mg	99.50 $\pm$ 0.0709

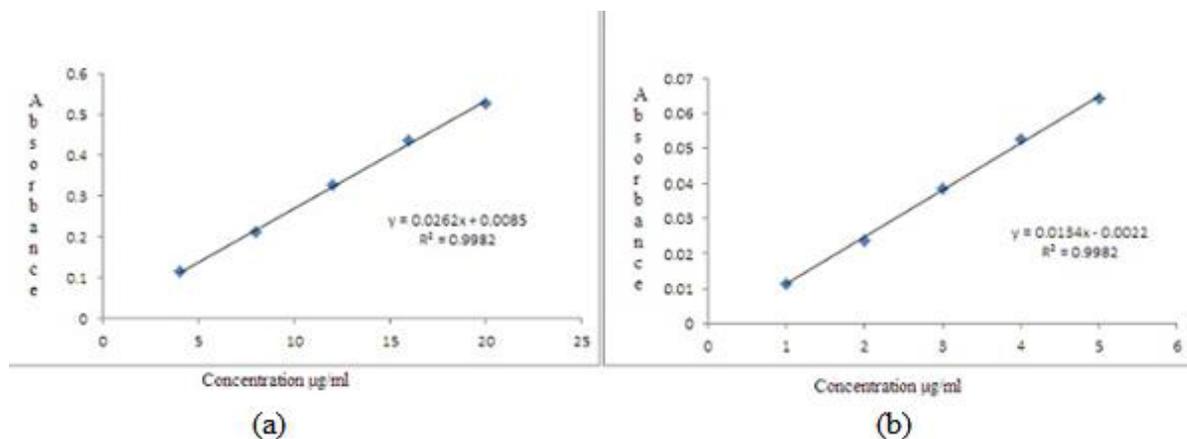
\*Mean value of three determination.

## RESULTS AND DISCUSSION

Developed spectrophotometric methods for the simultaneous estimation of CLZ and TLM were validated according to ICH guidelines and data complying with the standards were obtained. The results of validation parameters for all the three developed methods are reported (**Tables 1 and 4**).

## CONCLUSION

Three Spectrophotometric methods (Simultaneous equation method, absorbance ratio method, dual wavelength method) were developed for simultaneous estimation of CLZ and TLM in their combined formulation without prior separation (**Figure 8**). Methods were found to be precise and accurate as can be reflected from validation data. From the accuracy study and the value of LOD and LOQ it can be concluded that Simultaneous estimation method gives the best result for simultaneous estimation of Cilostazol and Telmisartan. Developed methods were successfully applied for estimation of CLZ and TLM in synthetic mixture and there for method is can be extended for the analysis of formulation.



**Figure 8.** Calibration curve for (a) CLZ 4-20 µg/ml (b) TLM 1-5 µg/ml (dual wavelength method).

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