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SIMULTANEOUS ESTIMATION OF ATORVASTATIN AND CLOPIDOGREL BY SIMULTANEOUS EQUATION METHOD IN CAPSULE DOSAGE FORM

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ABSTRACT

*The current work defines simple, precise, and economical method established on UV spectrophotometric technique for the simultaneous estimation of Atorvastatin (ATR) and Clopidogrel (*CP) in capsule dosage form. *The solvent used was methanol and the absorption maxima(λ_{max}) for Atorvastatin & Clopidogrel was found to be 246nm &202nm respectively. The calibration curve of was linear over the range of 5-25µg/ml for both Atorvastatin and Clopidogrel. The method was then validated for different parameters like accuracy, precision, and linearity as per ICH guidelines. The method showed good reproducibility and recovery with % RSD in the favorable range. This method can be effectively employed for the day to day analysis of both drugs in the capsule dosage form.*

Keywords *– Atorvastatin, Clopidogrel, Simultaneous equation method, ICH Guidelines.*

1. INTRODUCTION

Atorvastatin is a derivative of pyrrole and heptanoic acid and is an Inhibitor Hydroxymethyl glutaryl-Coa Reductase. Hence is used as Antihyperlipidemic Agent to reduce serum levels of LDL-Cholesterol; Apolipoprotein B; And Triglycerides. to prevent Cardiovascular diseases in patients with multiple risk factors by increasing the serum levels of HDL-Cholesterol.

Clopidogrel is a platelet aggregation inhibitor which is used in the patients with the risk of stroke and myocardial infraction in patients suffering from atherosclerosis. Clopidogrel has been associated with rare instances of distinctive, clinically apparent acute liver injury.

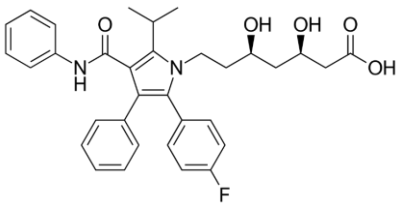

Fig. 1: Atorvastatin

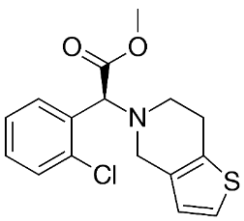

Fig. 2: Clopidogrel

The combination of atorvastatin and clopidogrel is given for the treatment and management of primary hypercholesterolemia, combined hyperlipidemia, heterozygous or homozygous familial hypercholesterolemia. Atorvastatin + Clopidogrel is also prescribed to reduce the atherosclerotic events in patients with history of recent MI or peripheral arterial disease or stroke.

The objective of the current research work was to develop a novel selective and validated spectrophotometric determination of Atorvastatin and Clopidogrel using methanol as a solvent in pharmaceutical dosage form [1-6].

2. MATERIALS AND METHODS

2.1 Instrumentation

The instrument used to measure the absorbance of the working solutions was a Shimadzu UV-Visible spectrophotometer of model UV 1700 with UV probe software. A Shimadzu AW120 Digital analytical balance and Equitron ultra sonicator were used in the study.

2.2 Method development

2.2.1 Selection of solvent: Different solvents were used to test the solubility of ATR and CP. Both the drugs were soluble in methanol but partially soluble in solvent like water and ethanol. So, methanol was selected as the solvent for this work.

2.2.2 Determination of Absorption maxima

Standard solution of Atorvastatin and Clopidogrel

100mg of Atorvastatin and Clopidogrel was precisely weighed and transferred into two clean and dry 100mlvolumetric flask separately then methanol was added to produce 100ml of stock solution to get a concentration of 1000 µg/mL.

5mL of stock was transferred into 100mL volumetric flask and the volume was made upto100mLto get a sub-stock of 50 µg/mL.

Determination: For determination of λ_{max} of both the drugs working solutions was prepared by pipetting out 1mL sub-stock into two 10mL volumetric flask and scanned in spectrum mode in the UV range of 200 ηm to 400 nm. Both ATR and CP showed the maximum absorbance at246ηm and 202 ηm respectively. AS shown in Fig.3.

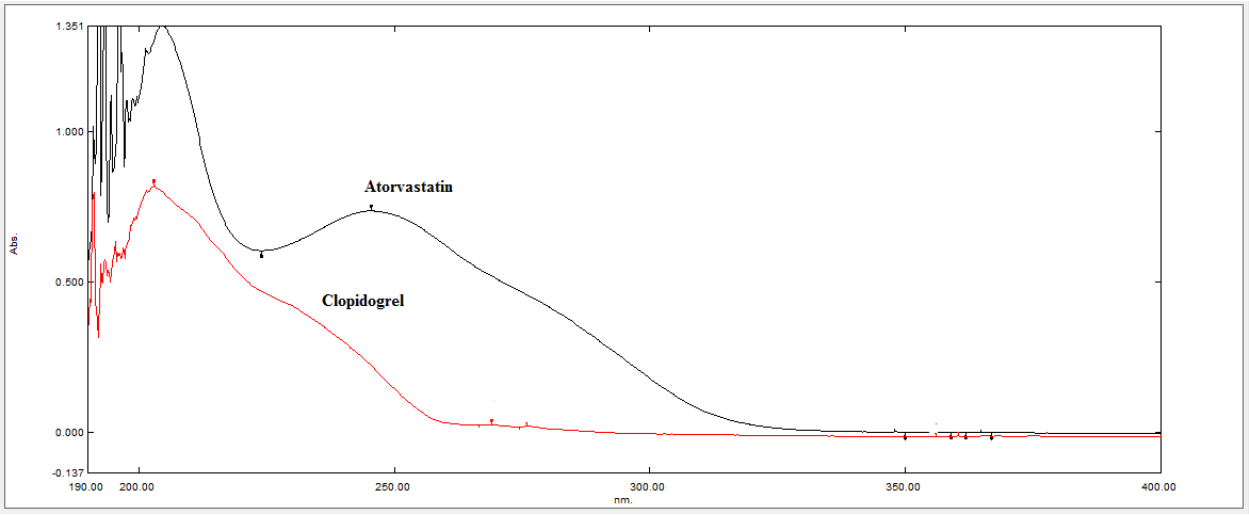

Fig.3: Overlay Spectrum of Atorvastatin and Clopidogrel

2.2.3 Preparation of calibration curve

Working standard solutions

The sub-stock solutions of both ATR and CP of volume 1, 2, 3, 4 & 5mL was further pipetted out to separate 10mL volumetric flask and diluted to 10mL to get the concentrations of 5, 10, 15, 20 and 25 µg/mL respectively.

Calibration curve of ATR and CP

The wavelength selected to measure the absorbance of both working standard was 246 nm and 202 nm. Methanol was used as the blank. Later calibration curve was plotted by taking respective absorbance versus concentration (µg/ml) & the regression equation was found out.

2.2.4 Simultaneous Equation Method

If a sample contains two absorbing drugs, each of which absorbs at the λmax of the other, it may be possible to determine both drugs simultaneously using multicomponent analysis UV Spectrophotometric 'Simultaneous Equation Method.

Two simultaneous equations (in two variables Cx and Cy) were formed using these Absorptivity coefficient values. Concentrations in the sample were obtained by using following equations

$$CX = A2ay1 - A1ay2 / ax1ay2 - ax2ay1 \ldots\ldots (1)$$

$$Cy = A1ax1 - A2ax1 / ax1ay2 - ax2ay1 \ldots\ldots (2)$$

Where, A1 and A2 are absorbance's of mixture at 246 nm and 202 nm respectively, ax1 and ax2 are absorptivity's of Atorvastatin at 2 wavelengths respectively and ay1 and ay2 are absorptivity's of Clopidogrel at 2 wavelengths, respectively. Cx and Cy are concentrations of Atorvastatin and Clopidogrel, respectively.

2.2.5 Sample solution preparation

Twenty capsules were taken, and the contents of the capsule was emptied into a mortar. Then the contents were finely crushed using a pestle and the weight of the powder is noted. The quantity of the powder equivalent to 100 mg of ATR was transferred to a 100 mL volumetric flask and filtered through Whatman filter paper. Required concentration of 5µg/ml of both the drugs was made by making necessary dilutions with methanol.

2.3 Validation of method

The current method was validated according to the parameters of ICH guidelines like linearity, accuracy, intraday and interday precision, LOD and limit of quantitation LOQ and robustness.

2.3.1 Range and linearity

The standard solution of 5 different concentration of ATR and CP, ranging between 5-25 µg/ml was prepared to study the linearity of both the drugs. Linearity was evaluated in terms of slope and intercept.

2.3.2 Precision

Precision is defined as the closeness of the readings obtained by multiple measurement of the same sample under prescribed conditions. Intraday and interday precision are considered for the precision studies.

1) Intraday Precision

The absorbance of the sample solutions of ATR and CP at the concentrations of 5, 15 and 25 µg/mL was measured three times on the same day and % R.S.D. was found out.

2) Interday Precision

The absorbance of the sample solutions of ATR and CP at the concentrations of 5, 15 and 25 µg/mL was measured on three alternative days and % R.S.D. was found out.

2.3.3 Accuracy

Accuracy is the closeness of the measured value to the actual value. Accuracy of the method was determined by carrying out the recovery studies. The recovery studies are carried out by spiking the previously analyzed sample solution of formulation with the standard drug solution**.**

2.3.4 Limit of Detection (LOD)

According to ICH guidelines LOD can be calculated by the following equation.

$$\mathbf{LOD = 3.3 \times (N / S)}$$

2.3.5 Limit of Quantification (LOQ)

According to ICH guidelines LOQ can be calculated by the following equation.

$$\mathbf{LOQ = 10 \times (N / S)}$$

Where, S is the slope of calibration curve and N is the standard deviation of peak areas.

2.3.6 Robustness and Ruggedness

Robustness is the capacity of an analytical method to give satisfactory results by small, deliberate variations in method parameters.

3. RESULTS AND DISCUSSION

The solutions of ATR and CP in a concentration range of 5 µg/ml was scanned in a range of 400-200 nm to determine the wavelength for the estimation. The maximum absorbance of ATR and CP was obtained at 246 nm and 202 nm, respectively. It is shown in Figure-3.

3.1 Method Validation

3.1.1 Linearity & Range

The linearity of both ATR and CP was found to be in the range of 5-25 µg/ml. The data for linearity ATR and CP is shown in Table 1 and 2, respectively.

Table 1: Linearity data of ATR

Drug	Conc. µg/ml	Absorbance	
		246 nm	202 nm
ATR	5	0.236	0.316
	10	0.424	0.632
	15	0.585	0.965
	20	0.858	1.29
	25	1.113	1.44

Table 2: Linearity data of CP

Drug	Conc. µg/ml	Absorbance	
		202 nm	**246 nm**
CP	5	0.345	0.064
	10	0.616	0.136
	15	0.867	0.188
	20	1.145	0.251
	25	1.346	0.289

3.1.2 Precision

1) Intraday Precision

The intraday precision of ATR and CP in terms of %RSD was found to be0.635-0.740% and 0.632-0.741% for ATR at 246 and 202 nm and 0.635-0.74% and 0.911-1.32% for CP at 202 nm and 246 nm, respectively.

2) Interday Precision

The intraday precision of ATR and CP in terms of % RSD was found to be0.72-1.06 % and 0.71-0.972% for ATR at 246 and 202nm 0.72 -1.056% and 0.965-1.45% for CP at 202 and 246 nm, respectively.

3.1.3 LOD and LOQ

The value of LOD for ATR and CP at 246 nm was found to be 0.5 µg/ml and 2.27 µg/ml, respectively.

The value of LOQ for ATR and CP at 246 nm was found to be 1.52 µg/ml and 6.87 µg/ml, respectively.

The value of LOD for ATR and CP at 202 nm was found to be 1.02 µg/ml and 3.16 µg/ml, respectively.

The value of LOQ for ATR and CP at 202 nm was found to be 3.1 µg/ml and 9.58 µg/ml, respectively.

3.1.4 Accuracy

The ability of the method to give accurate results was evaluated by recovery studies after spiking the marketed formulation at 50%, 100%, and 150% with the standard drug solution. The % recovery of ATR and CP was found to be in the range of 99.27-100.29% and 99.25-100.11%. The recovery studies data of ATR and CP is shown in Table. 3 and 4, respectively.

Table 3: Data for Recovery studies of ATR

Level of Recovery	Amount of Formulation	Amount of Pure Drug	Total Amount of Drug	Abs.	Diff.	% Recovery	Mean
50	10	5	15	0.665	0.429	98.85	100.99
				0.672	0.436	100.46	
				0.669	0.433	99.77	
100		10	20	0.861	0.437	100.69	99.27
				0.859	0.435	100.23	
				0.867	0.443	102.07	
150		15	25	1.021	0.436	100.4	99.67
				1.015	0.43	99.08	
				1.012	0.427	98.39	

Table 4: Data for Recovery studies of CP

Level of Recovery	Amount of Formulation	Amount of Pure Drug	Total Amount of Drug	Abs.	Diff.	% Recovery	Mean
50	10	5	15	0.975	0.63	100.8	99.57
				0.958	0.613	98.08	
				0.972	0.627	100.32	
100		10	20	1.23	0.614	98.24	99.25
				1.236	0.62	99.2	
				1.243	0.627	100.32	
150		15	25	1.489	0.622	99.52	100.11
				1.491	0.624	99.84	
				1.498	0.631	100.96	

3.1.5 Robustness

Robustness of the method was confirmed out by scanning both ATR and CP at two different wavelengths other than the λ_{max} of both the drugs and the % RSD was found to be 0.82-0.93 and 0.64-0.71 for ATR and CP respectively. Robustness study is shown in table 5 & 6.

Table 5: Robustness studies for Atorvastatin

Wavelength	Conc.	Absorbance		Wavelength	Conc.	Absorbance
243	15	0.572		249	15	0.587
		0.581				0.598
		0.574				0.593
	Average	0.575667			Average	0.592667
	ST DEV	0.004726			ST DEV	0.005508
	%RSD	0.820929			%RSD	0.929286

Table 6: Robustness studies for Clopidogrel

Wavelength	Conc.	Absorbance	Wavelength	Conc.	A
204	15	0.858	206	15	0.855
		0.853			0.867
		0.864			0.859
	Average	0.858333		Average	0.860333
	ST DEV	0.005508		ST DEV	0.00611
	%RSD	0.641659		%RSD	0.710202

3.1.6 Ruggedness

Ruggedness studies was carried out by changing the analyst and by changing the instrument for both the drugs by scanning each concentration three times at both the wavelengths and later the % RSD was found out. Ruggedness studies data is shown in table 7, 8, 9 & 10.

Table 7: Ruggedness studies of ATR at 246 nm

Drug	Wavelength	C	T1	T2	Mean	SD	%RSD
ATR	246nm	By changing the analyst					
		0	0	0	0	0	0
		5	0.232	0.235	0.2335	0.002121	0.9084884
		10	0.415	0.419	0.417	0.002828	0.6782799
		15	0.573	0.565	0.569	0.005657	0.9941747
		20	0.847	0.853	0.85	0.004243	0.4991342
		25	1.107	1.119	1.113	0.008485	0.7623793
		By changing the instrument					
		0	0	0	0	0	0
		5	0.23	0.232	0.231	0.001414	0.6122137
		5	0.416	0.412	0.414	0.002828	0.683195
		15	0.571	0.575	0.573	0.002828	0.4936173
		20	0.846	0.853	0.798	0.00495	0.6202691
		25	1.11	1.102	1.106	0.005657	0.5114696

Table 8: Ruggedness studies of ATR at 202 nm

Drug	Wavelength	C	T1	T2	Mean	SD	%RSD
CP	202nm	By changing the analyst					
		0	0	0	0	0	0
		5	0.321	0.324	0.3225	0.002121	0.657774
		10	0.639	0.645	0.642	0.004243	0.660847
		15	0.971	0.982	0.9765	0.007778	0.796536
		20	1.35	1.366	1.358	0.011314	0.833116
		25	1.52	1.532	1.526	0.008485	0.556047
		By changing the instrument					
		0	0	0	0	0	0
		5	0.319	0.323	0.321	0.002828	0.88113
		10	0.637	0.644	0.6405	0.00495	0.772794
		15	0.979	0.986	0.9825	0.00495	0.503791
		20	1.42	1.431	0.798	0.007778	0.974709
		25	1.528	1.516	1.522	0.008485	0.557509

Table 9: Ruggedness studies of CP at 202 nm

Drug	Wavelength	C	T1	T2	Mean	SD	%RSD
CP	202nm	By changing the analyst					
		0	0	0	0	0	0
		5	0.065	0.066	0.0655	0.000707	1.0795523
		10	0.134	0.136	0.135	0.001414	1.0475656
		15	0.186	0.183	0.1845	0.002121	1.1497671
		20	0.249	0.253	0.251	0.002828	1.1268634
		25	0.291	0.287	0.289	0.002828	0.9786945
		By changing the instrument					
		0	0	0	0	0	0
		5	0.061	0.062	0.0615	0.000707	1.1497671
		10	0.137	0.135	0.136	0.001414	1.0398629
		15	0.187	0.19	0.1885	0.002121	1.1253689
		20	0.251	0.258	0.798	0.00495	0.6202691
		25	0.295	0.291	0.293	0.002828	0.9653335

Table 10: Ruggedness studies of CP at 246 nm

Drug	Wavelength	C	T1	T2	Mean	SD	%RSD
CP	246nm	By changing the analyst					
		0	0	0	0	0	0
		5	0.345	0.35	0.3475	0.003536	1.01742
		10	0.616	0.624	0.62	0.005657	0.912396
		15	0.867	0.878	0.8725	0.007778	0.891481
		20	1.145	1.158	1.1515	0.009192	0.798297
		25	1.346	1.33	1.338	0.011314	0.845569
		By changing the instrument					
		0	0	0	0	0	0
		5	0.341	0.337	0.339	0.002828	0.834344
		10	0.623	0.629	0.626	0.004243	0.677738
		15	0.875	0.866	0.8705	0.006364	0.73107
		20	1.152	1.161	0.798	0.006364	0.797489
		25	1.345	1.335	1.34	0.007071	0.527692

Table 11: Analysis of marketed formulation

Tablet	Drug	Label Claim (mg)	Amount found	% label claim
Clopitorva 10	Atorvastatin	10	9.68	96.8
	Clopidogrel	75	73.32	97.76

Table 12: Regression Analysis data and Summary of Validation Parameters for the current method

Parameters	Atorvastatin		Clopidogrel	
Wavelength (nm)	246	202	202	246
Beer`s law limit (µg/ml)	5-25	5-25	5-25	5-25
Regression equation (y=mx+c)	y = 0.0434x - 0.0063	y = 0.0597x + 0.027	y = 0.0536x + 0.0498	y = 0.0118x + 0.0077
Slope (m)	0.0434	0.0597	0.0536	0.0118
Intercept (c)	0.0063	0.027	0.0498	0.0077
Correlation Coefficient (R^2)	0.9934	0.9913	0.9931	0.9946
Intraday (n=3) (%RSD)	0.635-0.740%	0.632-0.741%	0.635-0.74%	0.911-1.32%
Interday (n=3) (%RSD)	0.72-1.06 %	0.71-0.972%	0.72 -1.056%	0.965-1.45%
LOD(µg/ml)	0.5 µg/ml	1.02 µg/ml	3.16 µg/ml	2.27 µg/ml
LOQ(µg/ml)	1.52µg/ml	3.1 µg/ml	9.58 µg/ml	6.87µg/ml

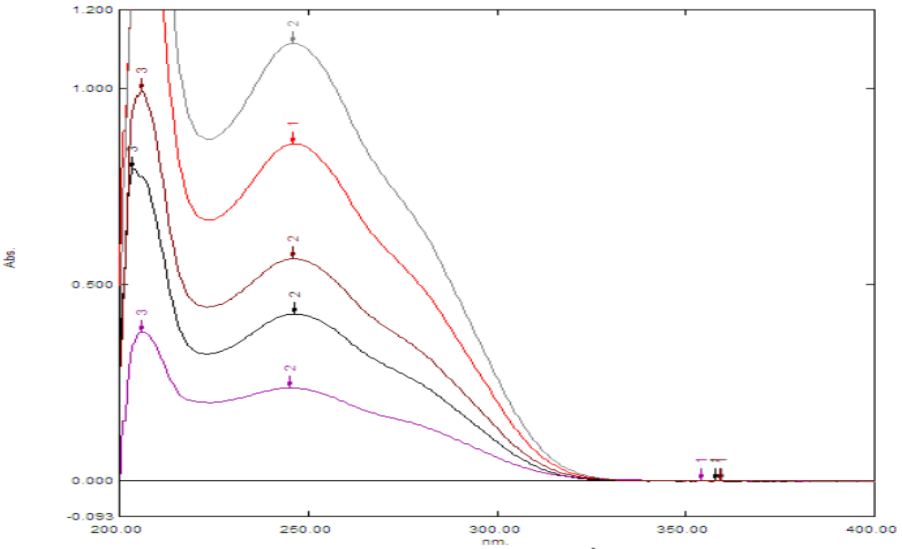

Fig. 4: Overlay Spectra of ATR (5-25 µg/mL)

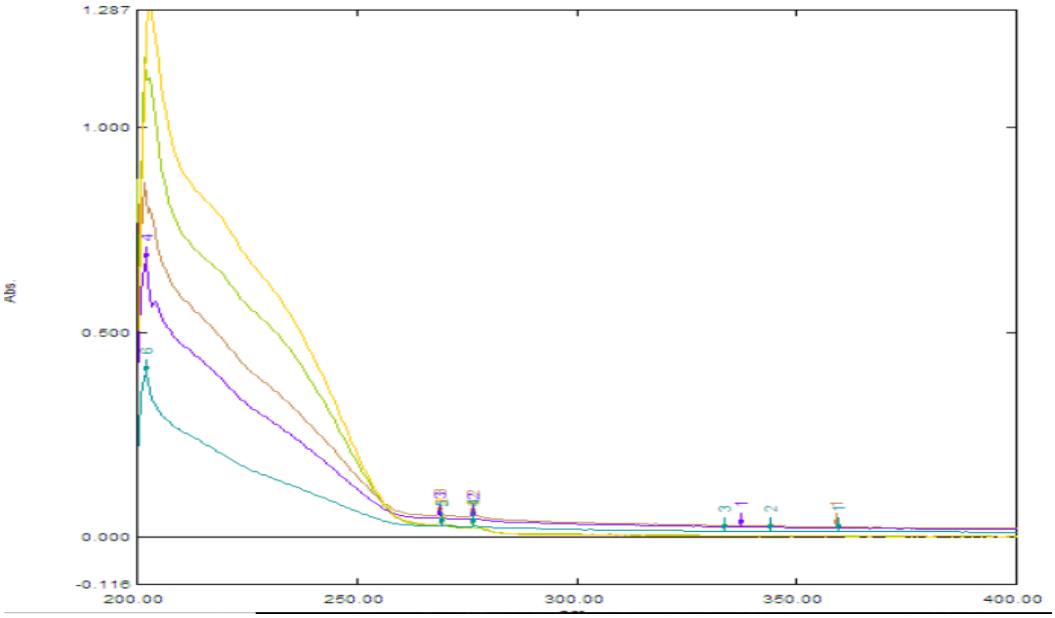

Fig. 5: Overlay Spectra of CP (5-25 µg/mL)

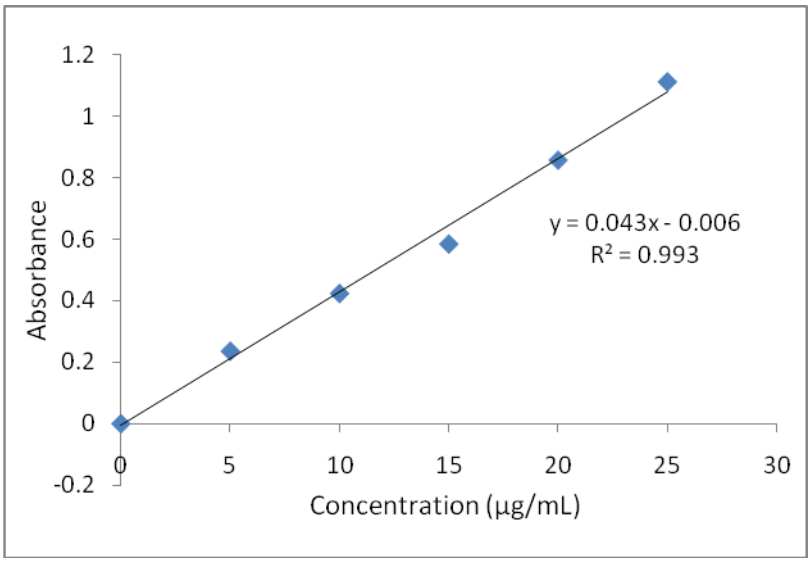

Fig. 6: Calibration curve of ATR at 246 nm

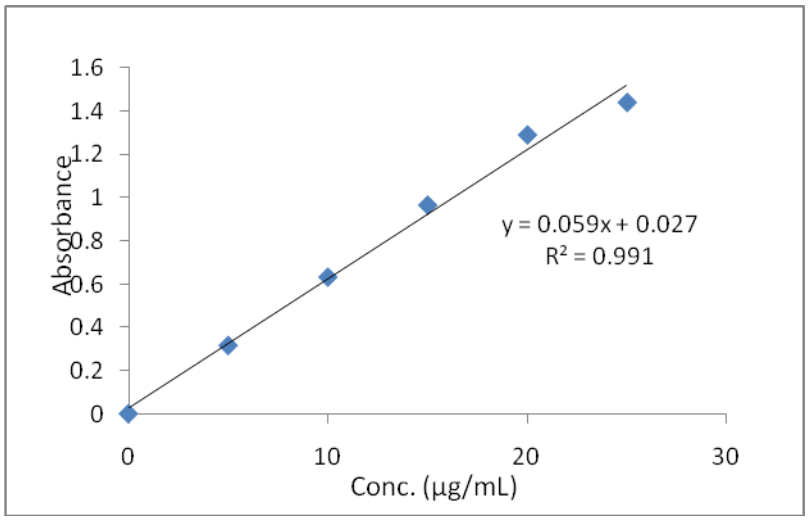

Fig. 7: Calibration curve of ATR at 202 nm

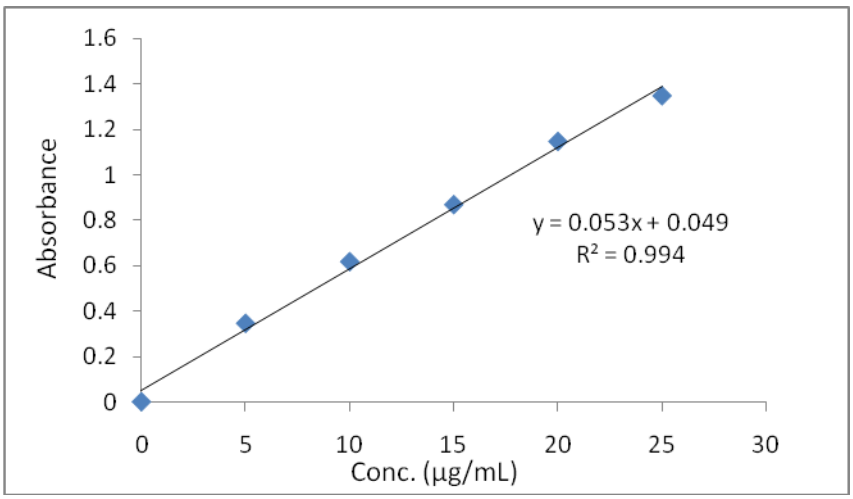

Fig. 8: Calibration curve of CP at 202 nm

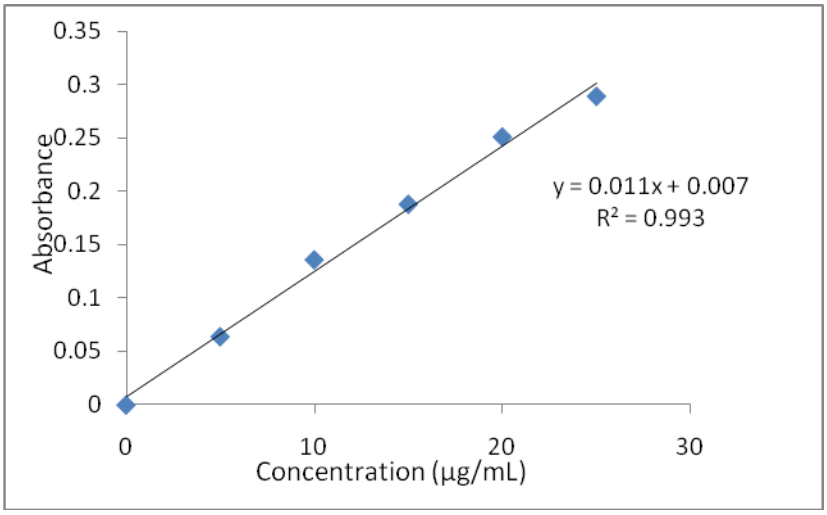

Fig. 9: Calibration curve of CP at 246 nm

4. CONCLUSION

A Novel, simple, sensitive, and economic UV spectrophotometric method has been developed for the routine analysis of Atorvastatin and Clopidogrel in capsule dosage form. This method is suitable for the simultaneous analysis of ATR and CP in multi-component formulation without the interference of one another. This method is recommended for the routine quality control analysis of the marketed formulation.

5. ACKNOWLEDGEMENTS

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