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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 is intended towards the development of a simple, rapid and precise UV-Visible spectrophotometric method for the simultaneous estimation of Atorvastatin (ATR) and Clopidogrel (CP) present in the marketed formulation using UV simultaneous equation. Methanol was used as a solvent and the absorbance of the drug was measured at the absorbance maxima (λ_{max}) of Atorvastatin & Clopidogrel, UV 246 nm & 202 nm respectively. Calibration curve plotted in concentration range 5-25 $\mu\text{g/mL}$ exhibited an excellent linear relationship for both Atorvastatin and Clopidogrel. The method was found to comply all the validation parameters as per the ICH guideline, such as accuracy (mean recovery = 99.97%), precision, and linearity ($r^2 = 0.9934$). The method showed good reproducibility and recovery with % RSD in the favorable range indicating the sensitivity of the method towards analyte. This method can be effectively employed for the day to day analysis of both drugs in the capsule dosage form.

INTRODUCTION: Atorvastatin **Fig. 1** is a derivative of pyrrole and heptanoic acid and is an Inhibitor Hydroxymethylglutaryl-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-Cholesterol¹⁻³. Clopidogrel **Fig. 2** is a platelet aggregation inhibitor which is used in the patients with the risk of stroke and myocardial infarction in patients suffering from atherosclerosis. Clopidogrel has been associated with rare instances of distinctive, clinically apparent acute liver injury^{2,4}.

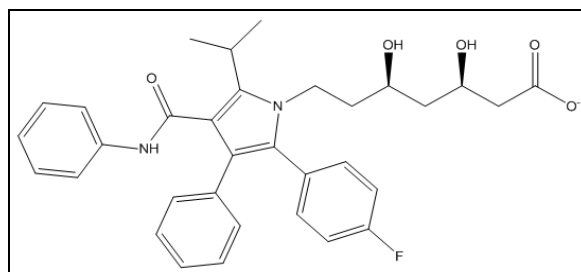


FIG. 1: ATORVASTATIN

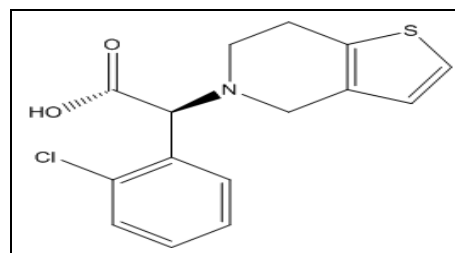


FIG. 2: CLOPIDOGREL

The literature review showed there are some HPLC methods⁵⁻¹⁰, UV and spectrophotometric methods¹¹⁻¹³, HPTLC¹⁴ method and electrophoresis method¹⁵ for the estimation of ATR and CP.

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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 a history of recent MI or true peripheral arterial disease or stroke¹⁻⁴.

OBJECTIVE: The current work objective is to develop a novel-selective and validated spectrophotometric determination of Atorvastatin and Clopidogrel using methanol as a solvent in the pharmaceutical dosage form.

MATERIALS AND METHOD:

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.

Method Development:

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 a solvent like water and ethanol. So, methanol was selected as the solvent and diluent for this work.

Determination of Absorption Maxima:

Standard Solution of Atorvastatin and Clopidogrel: 100 mg of Atorvastatin and Clopidogrel was precisely weighed and transferred into two clean and dry 100 ml volumetric flask, methanol was added to produce 100 ml of stock solution to get a concentration of 1000 µg/mL. 5 mL of the stock was transferred into 100 mL volumetric flask, and the volume was made up to 100 mL to get a sub-stock of 50 µg/mL.

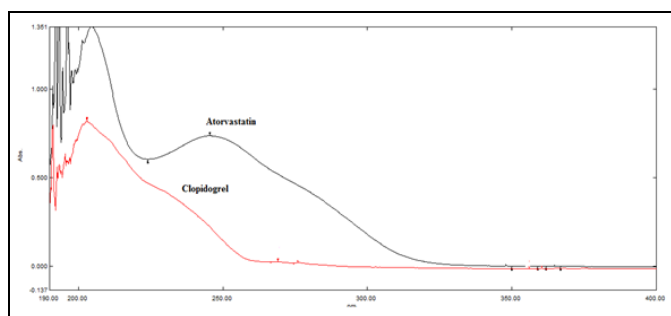


FIG. 3: OVERLAY SPECTRUM OF ATORVASTATIN AND CLOPIDOGREL

Determination: For determination of λ_{max} of both the drugs working solutions were prepared by pipetting out 1 mL sub-stock into two 10mL volumetric flasks and scanned in spectrum mode in the UV range of 200 nm to 400 nm. Both ATR and CP showed the maximum absorbance at 246 nm and 202 nm respectively as shown in **Fig. 3**.

Preparation of Calibration Curve:

Working Standard Solutions: The sub-stock solutions of both ATR and CP of volume 1, 2, 3, 4 & 5 mL was further pipetted out to separate 10mL volumetric flask and diluted to 10 mL 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. A later calibration curve was plotted by taking respective absorbance v/s concentration (µg/ml) & the regression equation was found out.

Simultaneous Equation Method: If a sample contains two absorbing drugs, each of which absorbs at the λ_{max} 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 the following equations

$$C_X = \frac{A_2 a_{y1} - A_1 a_{y2}}{a_{x1} a_{y2} - a_{x2} a_{y1}} \dots \dots (1)$$

$$C_Y = \frac{A_1 a_{x1} - A_2 a_{x2}}{a_{x1} a_{y2} - a_{x2} a_{y1}} \dots \dots (2)$$

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

Sample Solution Preparation: The contents of twenty capsules 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 Whatmann filter paper. Required concentration of 5 µg/ml of both the drugs was made by making necessary dilutions with methanol.

Validation of Method: The current method was validated according to the parameters of ICH guidelines like linearity, accuracy, intraday and interday precision, LOD, LOQ and ruggedness^{15, 16}.

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 regarding slope and regression analysis.

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

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 % RSD was found out.

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 % RSD was found out.

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

Limit of Detection (LOD): According to ICH guidelines LOD can be calculated by the following equation.

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

Limit of Quantification (LOQ): According to ICH guidelines LOQ can be calculated by the following equation.

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

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

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

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 **Fig. 3**

Linearity and 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. The overlay spectra and calibration curves of ATR and CP is shown in **Fig. 4, 5, 6 and 7** 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

Precision:

Intraday Precision: The intraday precision of ATR and CP in terms of % RSD was found to be 0.635-0.740% 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.

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

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 LOD for ATR and

CP at 202 nm was found to be 1.02 µg/ml and 3.16 µg/ml respectively as depicted in **Table 8**.

Accuracy: Recovery studies evaluated the ability of the method to give accurate results 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	Absorbance	Difference	% recovery	Mean recovery (%)
50		5	15	0.665	0.429	98.85	100.99
				0.672	0.436	100.46	
				0.669	0.433	99.77	
100	10	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	Absorbance	Difference	% recovery	Mean recovery (%)
50		5	15	0.975	0.63	100.8	99.57
				0.958	0.613	98.08	
				0.972	0.627	100.32	
100	10	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	

Ruggedness: Ruggedness studies were 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 5** and **6**.

TABLE 5: RUGGEDNESS STUDIES OF ATR AT 246 nm

Drug	Wavelength	Conc. (µg/mL)	T1	T2	Mean	SD	% RSD
ATR	246 nm	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 6: RUGGEDNESS STUDIES OF CP AT 202 nm

Drug	Wavelength	Conc. (µg/mL)	T1	T2	Mean	SD	% RSD
By changing the analyst							
		0	0	0	0	0	0

CP	202 nm		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
			5	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	0.258	0.00495	0.6202691
			25	0.295	0.291	0.293	0.002828	0.9653335

TABLE 7: ANALYSIS OF MARKETED FORMULATION

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

TABLE 8: 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)	1.52 µg/ml	3.1 µg/ml	9.58 µg/ml	6.87 µg/ml

T1 = Trial 1; T2 = Trial 2; SD = Standard deviation; % RSD = Relative standard Deviation; n = number of samples

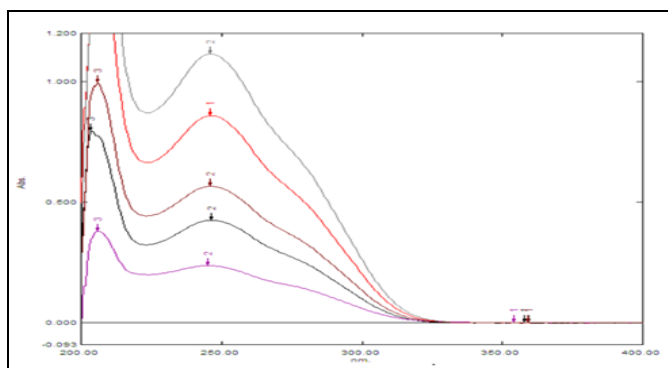


FIG. 4: OVERLAY SPECTRA OF ATR (5-25 µg/mL)

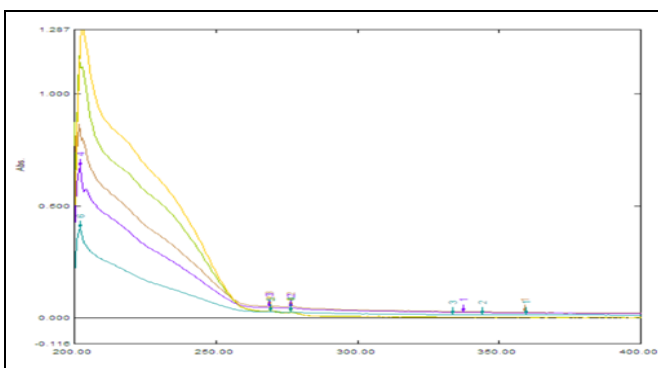


FIG. 5: OVERLAY SPECTRA OF CP (5-25 µg/mL)

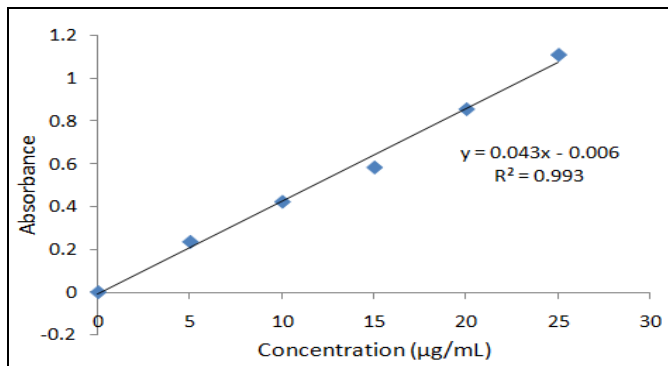


FIG. 6: CALIBRATION CURVE OF ATR AT 246 nm

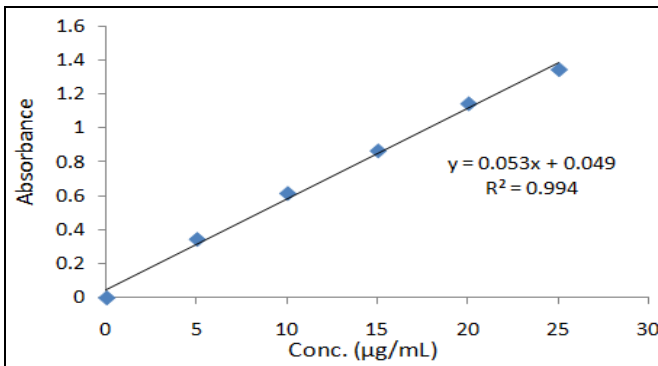


FIG. 7: CALIBRATION CURVE OF CP AT 202 nm

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

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CONFLICT OF INTEREST: The authors declare no conflict of interest for Current work.

REFERENCES:

1. Indian Pharmacopoeia, Vol. II, New Delhi: the controller of publications, Govt. of India 1996: 347
2. Goodman LS: Goodman and Gilman's the pharmacological basis of therapeutics. New York: McGraw-Hill 1996.
3. British Pharmacopoeia. Vol. 2 HMSO Publication centre, London 1993: 55.
4. US Pharmacopoeia, 23rd edition us Pharmacopoeial convention, Rockville MD 1995: 77.
5. Nagavi BJ and Gurupadaya BA: Simultaneous estimation of clopidogrel and atorvastatin in human plasma using bio-analytical RP-Ultra fast liquid chromatographic method. International Journal of Current Pharmaceutical Research 2015; 7(1): 30-5.
6. Londhe SV, Deshmukh RS, Mulgund SV and Jain KS: Development and validation of a reversed-phase HPLC method for simultaneous determination of aspirin, atorvastatin calcium and clopidogrel bisulphate in capsules. Indian Journal of Pharmaceutical Sciences 2011; 73(1): 23.
7. Panda SS: Ion-pairing RP-HPLC method for simultaneous determination of aspirin and clopidogrel bisulphate in tablet and capsule dosage form. Int J Pharm Tech Res 2010; 2: 269-73.
8. An K, Ayyappan T, Raman VR, Vetrichelvan T, Sankar AS and Nagavalli D: RP-HPLC analysis of aspirin and clopidogrel bisulphate in combination. Indian Journal of Pharmaceutical Sciences 2007; 69(4): 597.
9. Patel RB, Shankar MB, Patel MR and Bhatt KK: Simultaneous estimation of acetylsalicylic acid and clopidogrel bisulfate in pure powder and tablet formulations by high-performance column liquid chromatography and high-performance thin-layer chromatography. Journal of AOAC International 2008; 91(4): 750-5.
10. Peer CJ, Spencer SD, VanDenBerg DA, Pacanowski MA, Horenstein RB and Figg WD: A sensitive and rapid ultra HPLC-MS/MS method for the simultaneous detection of clopidogrel and its derivatized active thiol metabolite in human plasma. Journal of Chromatography B 2012; 880: 132-9.
11. Darwish HW, Hassan SA, Salem MY and El-Zeiny BA: Three different spectrophotometric methods manipulating ratio spectra for determination of binary mixture of Amlodipine and Atorvastatin. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 2011; 83(1): 140-8.
12. Kunturkar KL and Jain HK: Development and validation of UV spectrophotometric method for determination of s(-) metoprolol succinate and clopidogrel bisulphate in bulk and tablet dosage form. International Journal of Pharmacy and Pharmaceutical Sciences 2013; 5(3): 593-8.
13. Game MD, Gabhane KB and Sakarkar DM: Quantitative analysis of clopidogrel bisulphate and aspirin by first derivative spectrophotometric method in tablets. Indian Journal of Pharmaceutical Sciences 2010; 72(6): 825.
14. Londhe SV, Mulgund SV, Deshmukh RS and Jain KS: Simultaneous HPTLC analysis of aspirin, atorvastatin calcium and clopidogrel bisulphate in the bulk drug and capsules. Acta Chromatographica 2010; 22(2): 297-05.
15. AlShehri MM: A validated capillary electrophoresis method for simultaneous determination of ezetimibe and atorvastatin in pharmaceutical formulations. Saudi Pharmaceutical Journal 2012; 20(2): 143-8.
16. ICH, Q1A (R2) Stability testing of new drug substances and products in International Conference on Harmonization, Geneva Switzerland 2003.
17. ICH, Q2B Validation of Analytical Procedures: Methodology. International Conference on Harmonization, Geneva, Switzerland 1996.

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