WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 1, 1522-1532. Research Article ISSN 2277– 7105

DEVELOPMENT AND VALIDATION OF ABSORPTION RATIO METHOD FOR SIMULTANEOUS ESTIMATION OF ATORVASTATIN AND CLOPIDOGREL IN FORMULATION AND BULK

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Article Received on
05 Nov. 2019,

Revised on 26 Nov. 2019,
Accepted on 16 Dec. 2019,

DOI: 10.20959/wjpr20201-16512

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ABSTRACT

A simple, robust, precise, UV spectroscopic method has been developed for the simultaneous estimation of Atorvastatin and Clopidogrel in bulk and tablet dosage forms. In this paper the estimation of those drugs was carried out by absorbance ratio method. This method is based on measurement of absorption at 242nm and 232nm i.e, λ_{max} of Atorvastatin and Clopidogrel respectively. The linearity observed for Atorvastatin is in the range of 5-25 µg/ml and for Clopidogrel is in the range of 15-75 µg/ml. The accuracy of methods was assessed by recovery studies and was found to be within the range of 99.68%-99.75% for both Atorvastatin and Clopidogrel. The developed methods were validated with respect to linearity, accuracy (recovery), and precision. The method can be employed for estimation of pharmaceutical formulations with no interference from any excipients and diluents. The results were validated as per ICH guidelines.

KEYWORDS: Atorvastatin, Clopidogrel, Absorbance Ratio Method, ICH, Validation etc.

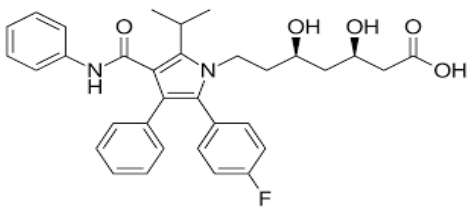

Fig. No. 1: Structure of Atorvastatin.

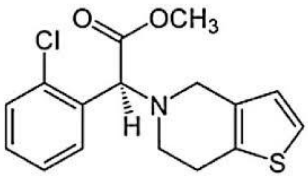

Fig. No. 2: Structure of Clopidogrel.

INTRODUCTION

Analytical Chemists ensure extremely important role in the advancement and development of pharmaceutical chemistry and science, especially in the industry. Analytical Chemists ensure the safety of products for human consumption.[1,2,3,4]

Atorvastatin reduces the production of cholesterol from the liver and reduces the risk of cardiovascular disease. Their combination is thus helpful in controlling dyslipidemia and reducing the risk of a heart. Clopidogrel is a prodrug of a platelet inhibitor used to reduce the risk of myocardial infarction and stroke. Atorvastatin is chemically known as [R-(R*, R*)]-2-(4-fluorophenyl)-β, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1Hpyrrole-1-heptanoic acid, calcium salt (2:1)trihydrate. Atorvastatinis a lipid-lowering drug belonging to the statin class of medications. By inhibiting the endogenous production of cholesterol within the liver, statins lower abnormal cholesterol and lipid levels and ultimately reduce the risk of cardiovascular disease. The clear evidence of the benefit of statin use coupled with very minimal side effects or long term effects has resulted in this class becoming one of the most widely prescribed medications in world.[4,5,6]

Clopidogrel is chemically known as methyl (2S)-2-(2-chlorophenyl)-2-(6,7-dihydro-4H-thieno[3,2-c]pyridin-5-yl)acetate;sulfuric acid. A prodrug of a platelet inhibitor used to reduce the risk of myocardial infarction and stroke. Clopidogrel is indicated to reduce the risk of myocardial infarction for patients with non-ST elevated acute coronary syndrome (ACS), patients with ST-elevated myocardial infarction, and in recent MI, stroke, or established peripheral arterial disease. It has been shown to be superior to aspirin in reducing cardiovascular outcomes in patients with cardiovascular disease and provides additional benefit to patients with acute coronary syndromes already taking aspirin.[4,5,6,7,8]

From the extensive literature review, few analytical methods were reported for analysis such as UV[9,10] HPLC[11,12,13,14,15] and HPTLC[16,17] and by other methods but there are very few analytical methods were reported in combination.

The challenges for the quality control and bioavailability of ATOR and CLOP in generics produced in India inspired the authors to develop simple methods for quantification of ATOR and CLOP in bulk and stability study in UV – Visible Spectrophotometer. The developed methods were validated according to International conference of harmonization (ICH) guidelines.[18,19,20]

MATERIALS AND METHODS

Instruments

Shimadzu UV-1800 double beam spectrophotometer was used to record the spectra of sample and reference solutions using pair of Quartz cells of 10mm path length. All weighing was carried out on Shimandzu AUX220 weighing balance. Sonicator of Ultra sonic is used for the purpose of sonication, Filter papers of Sartorius Stedim Biotech of grade 292 are used for the filtration purpose.

Chemicals

The bulk drug of Atorvastatin was provided by blue cross laboratory ltd, nashik, as gift sample and Clopidogrel was provided by cipla ltd India. Fixed dose combination tablet Aztolet 10, containing atorvastatin 10mg and clopidogrel 75mg was procured from local Market All chemicals and reagents of analytical grade and HPLC grade were purchased from USV LTD, Mumbai, India.

Preparation of stock solution and selection of wavelength

A) Atorvastatin Standard stock solution [A]

An accurately weighed quantity of ATOR (10 mg) was taken in 10 mL volumetric flask and dissolved in methanol (8 mL) with the help of ultrasonication for about 10 min. Then the volume was made up to the mark using methanol to get atorvastatin standard stock solution (1 mg / mL).

B) Atorvastatin Working Standard Solution [A_1]

ATOR standard stock solution [A] (1 mL) was diluted to 10 mL using 80% v/v methanol to get working standard solution (100 µg / mL).

C) Clopidogrel Standard Stock Solution [C]

An accurately weighed quantity of CLOP (75 mg) was taken in 10 mL volumetric flask and dissolved in methanol(8 mL) with the help of ultrasonication for about 10 min. Then the

volume was made up to the mark using methanol to get Clopidogrel standard stock solution (1 mg / mL).

D) Clopidogrel Working Standard Solution [C_1]

CLOP standard stock solution [C] (1 mL) was diluted to 10 mL using 20% v/v methanol aqueous solution to get working standard solution (100 µg / mL).

Determination of λ Max of Individual Component

An appropriate aliquot portion working standard of ATOR (0.5 mL) and CLOP(4.0 mL) were transferred to two separate 10 mL volumetric flasks, the volume was made up to the mark using 80% v/v methanol to obtain ATOR (8 µg/mL) and CLOP (75µg/mL). Drug solutions were scanned separately between 200 nm to 400 nm ATOR shows the λ_{max} at 242nm while isobestic point shows λ_{max} at 232nm for ATOR and CLOP as shown in fig No. 3.

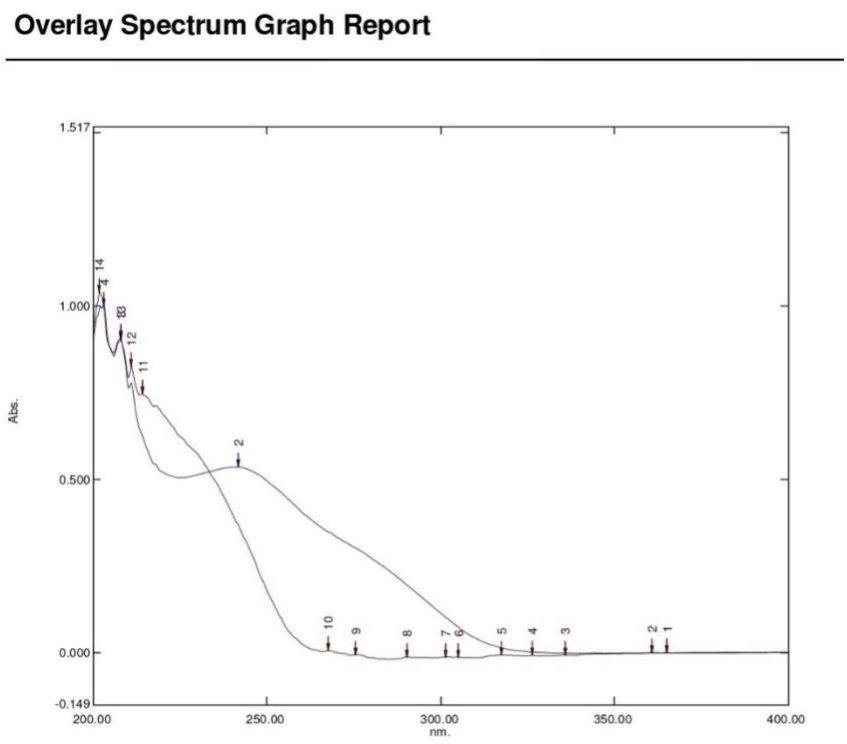

Fig. 3: An Overlay Spectra of Atorvastatin and Clopidogrel.

Linearity study for Atorvastatin

An accurately measured aliquot portion of working standard solution of ATOR was transferred to seven separate 10mL volumetric flasks. The volume was made up to the mark using 20% v/v aqueous methanol to obtain concentrations (5µg/ml, 10µg/ml, 15µg/ml,

20µg/ml,25µg/ml).Absorbance of these solutions was plotted as Absorbance vs concentration. The results are shown in Table No. 1.

Linearity study for Clopidogrel

Accurately measured aliquot portions of working standard solution of CLOP were transferred to seven separate 10 mL volumetric flasks. The volume was made up to the mark using 20% v/v aqueous methanol solution to obtain concentrations (15µg/ml, 30µg/ml, 45µg/ml, 60µg/ml, 75µg/ml). Calibration curve was plotted, absorbance vs concentration, absorbance measured at 242 nm. The results shown in the Table No. 1.

Table No. 1: Regression and Optical characteristics of ATOR and CLOP.

Parameters	Value for Atorvastatin	Value for Clopidogrel
Beer's law limit (µg/ml)	5-25 µg/ml	15-75 µg/ml
Regression Coefficient(R^2)	0.9980	0.9996
Regression equation	y= 0.051x +0.0152	y= 0.0069-0.0011
Slope	0.051	0.0069
Intercept	0.0152	0.0011

The study of regression and optical characteristics of ATOR and CLOP are carried out in which Regression Coefficient (R^2) of ATOR is 0.9980 and of CLOP is 0.9996. The slope of ATOR 0.051 and slope of CLOP is 0.0069 with Intercept of ATOR 0.0152 and for CLOP 0.0011 Therefore, Concentration vs Absorbance are fairly linear between both co-ordinates by statistical manner and obey ICH guidelines.

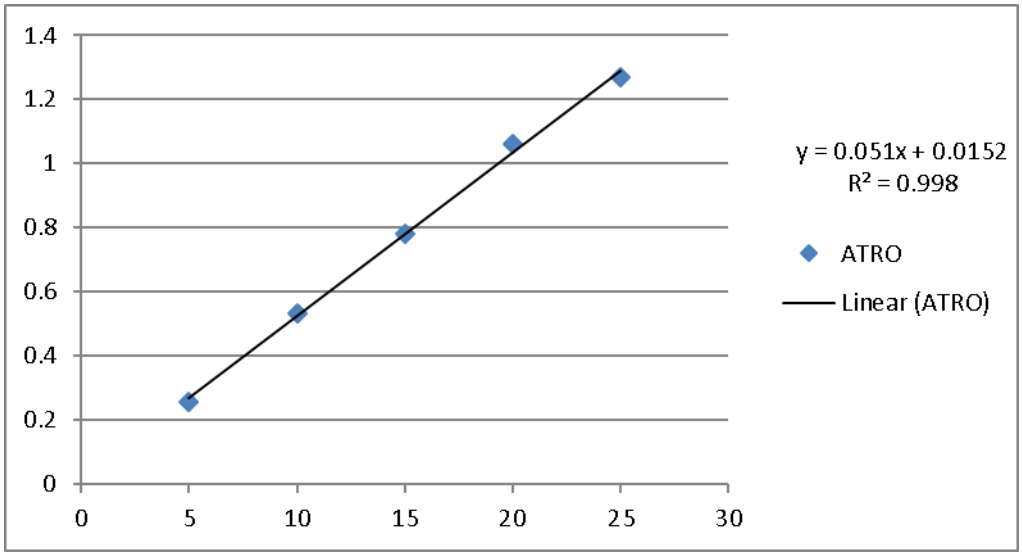

Fig. 4: Calibration curve of Atorvastatin at 242nm.

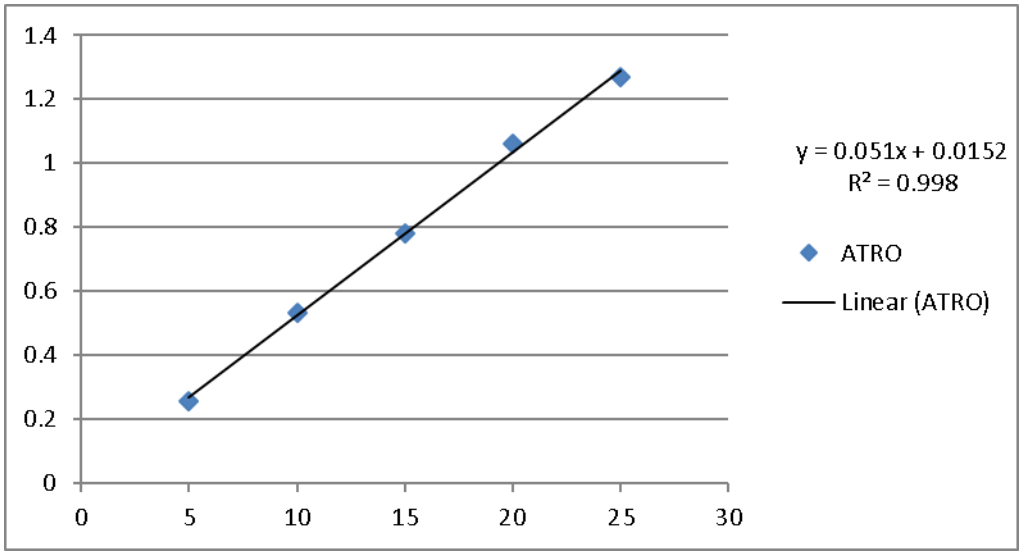

Fig. 5: Calibration curve of Clopidogrel at 232nm.

Estimation of Laboratory mixture by proposed method

The absorbance ratio method is a modification of the simultaneous equations procedure. It depends on the property that, for a substance which obeys Beer's Law at all wavelengths, the ratio of absorbance at any two wavelengths is a constant value independent of concentration or path length.

Absorbance method uses the ratio of absorbance at two selected wavelengths, one at isoabsorptive point and other being the λmax of one of the two drug Atorvastatin and Clopidogrel have λmax at 242 and 232 nm respectively and isoabsorptive point 242nm. The wavelengths selected for analysis were 242 and 232 nm, respectively. E (1%, 1cm) values of Atorvastatin and Clopidogrel were determined at 242and 232nm.

The concentration of two drugs in mixture was calculated by using following equations

$$Cx = \frac{Qm - Qy}{Qx - Qy} X \frac{A}{ax1} \quad (1)$$

$$Cy = \frac{Qm - Qx}{Qy - Qx} X \frac{A}{ay1} \quad (2)$$

Where,

$$Qm = \frac{\text{Absorbance of sample at 242 nm}}{\text{Absorbance of sample at 232 nm}}$$

$$Qx = \frac{\text{E (1 \% 1cm) of ATOR at 242 nm}}{\text{E (1 \% 1cm) of ATOR at 232nm}}$$

$$Qy = \frac{\text{E (1 \% 1cm) of CLOP at 242 nm}}{\text{E (1 \% 1cm) of CLOP at 232 nm}}$$

The results are displayed in the Table No. 2.

Table No. 2: Results of Estimation of ATOR and CLOP in standard laboratory mixture.

Analyte	% Concentration estimated (Mean ± S.D)	% R.S.D
Atorvastatin	99.81±0.08956	0.08345
Clopidogrel	99.79±0.073689	0.07465

The estimation of ATOR and CLOP in Standard Laboratory Mixture are carried out in which % concentration of ATOR and CLOP were found to be 99.81 and 99.79 respectively. Those values are fairly accurate by statistical manner and are as per ICH guidelines.

Application of proposed method for Estimation of drugs in tablet

Aztolet 10 tablet containing atorvastatin (10mg) and clopidogrel (75mg) contents was weighed and ground to fine powder. A quantity of sample equivalent to atorvastatin (10mg) and clopidogrel (75 mg) was transferred into 100 mL volumetric flask containing methanol (60 mL), sonicated for 15 min and the volume was made up to the mark and filtered through Whatman filter paper (No. 45). This solution was (1ml) transferred to 10 mL volumetric flaks, dissolved and volume was adjusted to the mark. The absorbances of the solutions were measured at 242 nm and 232 nm against blank. The concentrations of two drugs in sample were determined by using simultaneous equations**.** The results are reported in the Table No.3.

Table No. 3: Results of Estimation of ATOR and CLOPin tablet dosage form.

Analyte	Label claim(mg/cap)	% Label claim estimated (Mean±S.D)	% R.S.D
Atorvastatin	10	99.68± 0.0563	0.0584
Clopidogrel	75	99.75±0.0518	0.0118

The results of Estimation of ATOR and CLOP in tablet dosage shows form the % purity 99.68 to 99.75 with SD and RSD bellow 2 which is fairly accurate by statistical manner and are as per ICH guidelines.

Validation of proposed method

The proposed method was validated as per ICH guidelines.

Accuracy (Recovery study)

Accuracy of proposed method was ascertained on the basis of recovery study performed by standard addition method. A known amount of standard drug solutions were added to the tablet powder to make final concentrations in the range of 80%, 100% and 120% and re-analyzed it by the proposed method. The absorbance recorded and the % recoveries were calculated using formula.

% Recovery = [A - B/ C] X 100

Where,

A = Total amount of drug estimated

B = Amount of drug found on pre analysed basis

C = Amount of Pure drug added.

The results are reported in the Table No. 4

Table No. 4: Recovery study.

Drug in mixture solution (µg/ml)		% Recovery ± S.D.	
Atorvastatin	**Clopidogrel**	**Atorvastatin**	**Clopidogrel**
5	45	99.58 ± 0.817	99.76 ± 0.560
10	60	99.78 ± 0.855	99.64 ± 0.760
15	75	99.57 ± 0.306	99.54 ± 0.670

The results of Recovery study of ATOR and CLOP are found to be fairly accurate between 99.58 to 101.3% for ATOR and 99.64 to 100% for CLOP between various concentrations under observation by statistical way and are obey ICH guidelines.

Precision

Precision was determined as intra-day and inter-day variations. Intra-day precision was determined by analyzing Atorvastatin (5, 10, and 15 µg/mL) and Clopidogrel (45, 60, and 75 µg/mL) for three times on the same day. Inter-day precision was determined by analyzing the same concentration of solutions for three different days over a period of week. The results are shown in the Table No. 5.

Table No. 5: Precision study.

Drug	Conc. [µg/mL]	Intra-day Amount Found		Inter-day Amount Found	
		Mean ±S.D [*n* = 5]	% R.S.D.	Mean ± S.D. [*n* =5]	% R.S.D.
ATOR	5	4.82 ± 0.0871	0.8850	4.85 ± 0.08600	0.8855
	10	9.82 ± 0.2105	1.061	9.81± 0.2105	1.060
	15	14.93 ± 0.4625	1.159	14.87± 0.4622	1.157
CLOP	45	44.88 ± 0.1260	0.6354	44.85 ± 0.1190	0.6002
	60	59.84 ± 0.2592	0.6503	59.78 ± 0.3066	0.7686
	75	74.83 ± 0.080	0.839	74.78 ± 0.07396	0.7480

The Precision Study of ATO and CLO were carried out and Results are found to be fairly accurate by statistical manner as per ICH guidelines.

Ruggedness

Ruggedness of the proposed method was determined by analysis of aliquots from homogenous slot by two different analyst using same operational and environmental conditions. The results are reported in the Table No. 6.

Table No. 6: Ruggedness Study.

	Atorvastatin 10 µg/ml		Clopidogrel75 µg/ml	
	Amount found in µg/ml Mean ± S.D. (n=3)	% R.S.D	Amount found in µg/ml Mean ± S.D. (n=3)	% R.S.D
Analyst I	9.95± 0.2065	0.2585	75.85± 0.0953	0.8695
Analyst II	9.76 ± 0.4685	0.5878	75.95 ± 0.1671	1.8787
Day I	9.96 ± 0.2253	0.2818	74.01 ± 0.1083	1.0806
Day II	9.83 ± 0.5411	0.6782	74.90 ± 0.1213	1.2125
Instrument I	9.84 ± 0.1185	0.1485	74.995 ± 0.1259	1.2585
Instrument II	9.87± 0.1229	0.1535	75.023± 0.09645	0.9626

n= 3

The Ruggedness study of ATO and CLO are carried out and results are found to be fairly accurate by statistical manner and obeys ICH guidelines.

Limit of Detection

LOD: Limit of detection of Atorvastatin and clopidogrel was found to be 1.21714 µg /ml and 2.034761µg /ml respectively.

LOQ: Limit of Quantitation of was found to be Atorvastatin and clopidogrel 4.057134µg /ml and 6.165944µg /ml respectively.

RESULTS AND DISCUSSION

An Absorbance Ratio Method in UV Spectroscopy was developed for Atorvastatin and Clopidogrel. The method employs 242nm as λ1 and 232 nm as λ2 for formation of equations. Atorvastatin and Clopidogrel obeys Beer's law in the concentration range 5-25 µg/ml(R^2=0.998) and 15-75 µg/ml (R^2=0.9996) respectively. The mean recovery for Atorvastatin and clopidogrel was found to be 99.57 to 99.78% and 99.54 to 99.76%respectively. The developed method was validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values.

CONCLUSION

The proposed method can be used for the daily quality control of Atorvastatin and Clopidogrel in laboratories. All the authors hereby declare that there is no conflict of interest regarding the publication of this article.

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