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Development of a RP-HPLC Method for Simultaneous Determination of Atorvastatin Calcium and Clopidogrel Bisulphate in Pharmaceutical Formulation

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ABSTRACT

An isocratic reversed-phase liquid chromatograpic assay method was developed for the quantitative determination of Atorvastatin calcium (ATOR) and Clopidogrel bisulphate (CLOP) in combined dosage form. A Zodiac C_{18}, 250x4.6mm, 5µm column and OPA Buffer: ACN (70: 30, v/v) as mobile phase. The flow rate was 1mL/min and effluents were monitored at 241 nm. The retention times of Atorvastatin and Clopidogrel were 5.8 min and 3.5 min respectively. The correlation coefficient was found to be 0.99947 (for ATOR) and 0.99944 (for CLOP). The proposed method was validated with respect to linearity, accuracy, precision, and robustness. The method was successfully applied to the estimation of Atorvastatin and Clopidogrel in combined capsule dosage form.

Keywords: RP-HPLC, Atorvastatin, Clopidogrel, Formulation, Capsule, Validation.

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INTRODUCTION

Atorvastatin calcium is chemically [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. Atorvastatin is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis. Atorvastatin (ATOR) is anti-cholinergic drug used for the treatment of urinary urgency. Clopidogrel (CLOP) is chemically(+)-(*S*)-methyl2-(2-chlorophenyl)-2-(6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl) acetate. Clopidogrel is an oral, thienopyridine-class antiplatelet agent used to inhibit blood clots in coronary artery disease, peripheral vascular disease, cerebrovascular disease, and to prevent myocardial infarction (heart attack).At present, these drugs are available in combination therapy. Oral administration of Atorvastatin (ATOR) and Clopidogrel (CLOP) has been found to be more effective than use of either drug alone. Combination treatment with Atorvastatin and Clopidogrel effectively reduces disease.[1–5]

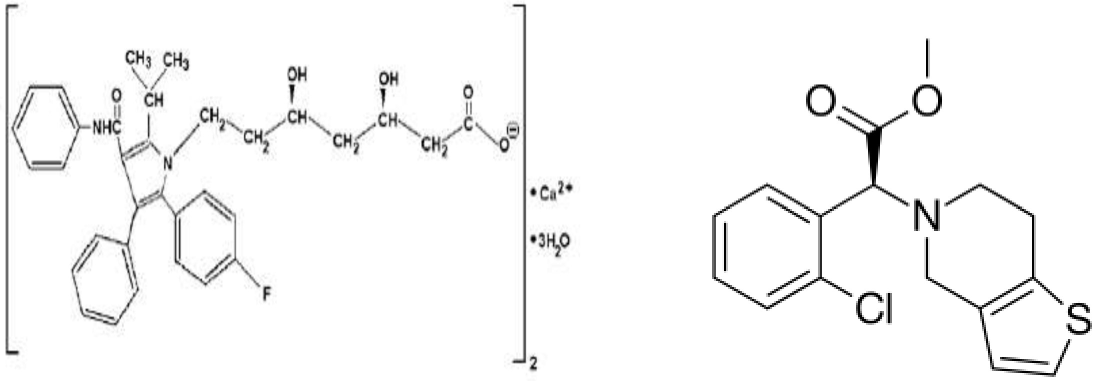

Figure 1: Structure of Atorvastatin (ATOR) and Clopidogrel (CLOP)

Literature survey revealed various spectrophotometric[6–9,] HPTLC[10,11] and HPLC[12–15] methods have been reported for estimation of ATOR individually or in combination with other drugs. Different spectrophotometric[16], HPTLC[19] and HPLC[17] methods have been reported for estimation of CLOP individually or in combination with other drugs. To the best of our knowledge, no analytical methods have been reported for analysis of ATOR and CLOP in pharmaceutical formulation. Hence the aim of the present study is to establish an accurate and sensitive RP-HPLC[17] method and, after validation in accordance with International Conference on Harmonization (ICH) guidelines, to use the method for analysis of the drug content of both in Capsule dosage form.[5]

MATERIALS AND METHODS

The Liquid chromatographic system consisted of Shimadzu- Model LC20AT, Spin chrome soft ware HPLC with variable wavelength programmable UV/VIS detector and Rheodyne injector with 20µL fixed loop. The analytes were monitored at 241nm. Chromatographic analysis was performed on zodiac C_{18} column having 250 × 4.6 mm, 5 µm particle size. All drugs and chemicals were weighed on Shimadzu electronic balance (AX 200, Shimadzu Corp., Japan).

Chemicals and Reagents

Analytically pure samples of ATOR and CLOP were obtained as a gift samples from Rainbow labs (Hyderabad, India), HPLC grade methanol obtained from E. Merck Ltd., Mumbai, India while analytical reagent grade methanol, Ortho phosphoric acid (OPA) obtained from Astron Chemicals, India. Capsule formulation containing labelled amount of 10 mg of ATOR and 75 mg of CLOP, were procured from local market.

Chromatographic conditions

A zodiac C_{18} (250×4.6 mm, 5µm) chromatographic column equilibrated with mobile phase OPA buffer: ACN (70:30, v/v) was used. Mobile phase flow rate was maintained at 1 mL/min and effluents were monitored at 241 nm. The sample was injected using a 20 µL fixed loop, and the total run time was 8 min.

Preparation of standard stock solutions

Mix Stock solution containing CLOP and ATOR was prepared in methanol having concentration 7500 µg/ mL CLOP and 1000 µg/ mL ATR. Aliquot of the standard solution was appropriately diluted with the mobile phase containing OPA buffer: ACN (70:30) to get the concentration of 75µg/mL for CLOP and 10µg/mL for ATOR respectively.

Preparation of Sample Solution:

An accurately weighed quantity of capsule powder equivalent to 75.0 mg of CLP and 10.0 mg of ATR was transferred to 100 mL volumetric flask, sonicated for 30 minutes with sufficient quantity of methanol and volume was made up to mark with methanol. The contents of the flask were filtered through Whatmann filter paper (no.41). A 5.0 mL portion of the filtrate was further diluted to 50.0 mL with a water and ACN (70:30) v/v.

Method Validation

The developed method was validated for various parameters like linearity, range, accuracy, precision, robustness, system suitability, specificity, LOQ and LOD.

Linearity and Range

Appropriate aliquots of ATOR and CLOP working standard solutions were taken in different 10 ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentration range of 1- 15 µg/ml for ATOR and 5-120 µg/ml for CLOP. The solutions were injected using a 20 µL fixed loop system and chromatograms were recorded. Calibration curves were constructed by plotting average peak area versus concentrations and regression equations were computed for both drugs.

Precision

The intra-day and inter-day precision studies were carried out by estimating the corresponding responses 3 times on the same day and on 3 different days for three different concentrations and the results were reported in terms of relative standard deviation. The instrumental precision studies were carried out by 3 different concentrations and results are reported in terms of relative standard deviation.

Accuracy

The accuracy of the method was determined by calculating recoveries of ATOR and CLOP by method of standard additions. Known amount of ATOR were added to a pre quantified sample solution and the amount of ATOR and CLOP were estimated by measuring the peak areas and by fitting these values to the straight-line equation of calibration curve.

Detection limit and Quantitation limit

The limit of detection (LOD) is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels. Limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated using the following equation as per ICH guidelines.

$LOD = 3.3 \times s/S$; $LOQ = 10 \times s/S$; Where s is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

Solution stability

Stability of sample and standard solution were stable up to 48 h at room temperature.

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities and degradation products. Commonly used excipients (starch, microcrystalline cellulose and magnesium stearate) were spiked into a pre weighed quantity of drugs. The chromatogram was taken by appropriate dilutions and the quantities of drugs were determined.

Robustness

Robustness of the method was studied by deliberately changing the experimental conditions such as flow rate, temperature and percentage of organic phase.

RESULTS AND DISCUSSION

Optimization of mobile phase

Various mixtures containing methanol, water and aqueous buffer were tried as mobile phases in the initial stage of method development. Finally, the system containing mixture of OPA buffer: ACN (70:30, v/v) was found to be satisfactory and gave two well-resolved peaks for ATOR and CLOP. The retention time for ATOR and CLOP were 5.8 min and 3.5 min, respectively (Figure 2). The resolution between ATOR and CLOP was found to be **2.0**, which indicates good separation of both the compounds. The mobile phase flow rate was maintained at 1 mL/min. Overlaid UV spectra of both the drugs showed that ATOR and CLOP absorbed appreciably at 241 nm, so detection was carried out at 250 nm.

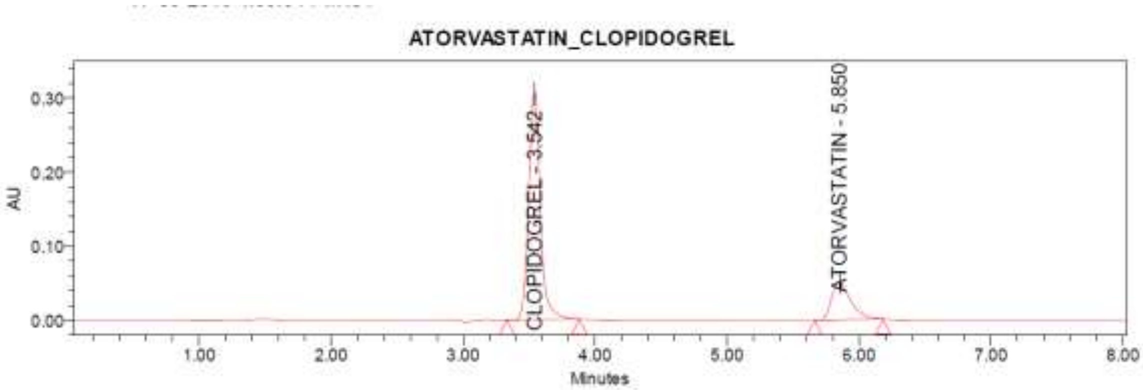

Figure 2: HPLC chromatogram of ATOR (RT 5.8 min) and CLOP (RT 3.5 min), using OPA buffer and ACN (70:30, v/v) as mobile phase.

Validation of the Proposed Methods

The developed method was validated for various parameters including linearity and range, accuracy, precision, robustness, system suitability, specificity, LOQ and LOD.

Linearity and Range

The calibration curve for ATOR was found to be linear in the range of 1-5 µg/ml with a correlation coefficient of 0.9991. The calibration curve for CLOP was found to be linear in the range of 5-120 µg/ml with a correlation coefficient of 0.9992.

The regression analysis of calibration curves are shown in Table 1.

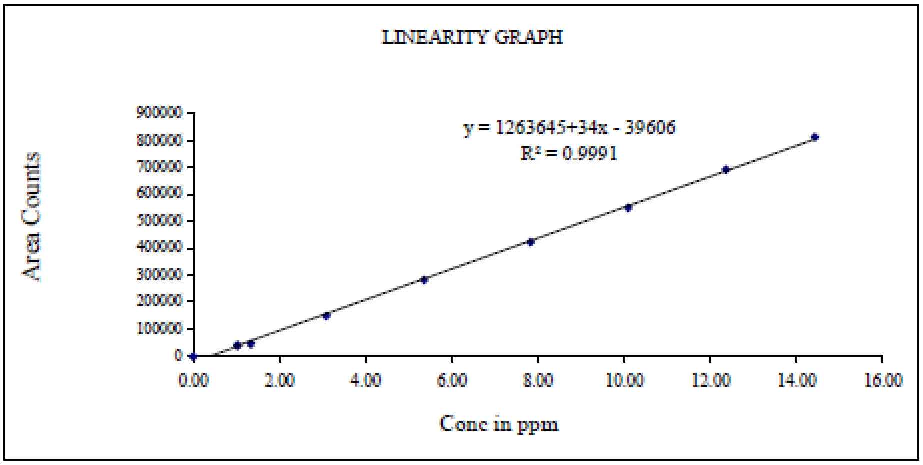

Figure 3: Calibration curve of ATOR

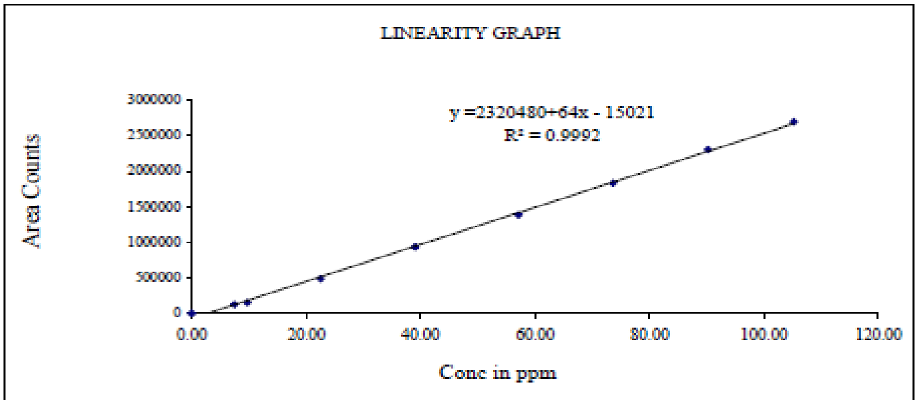

Figure 4: Calibration curve of CLOP

Table 1: Calibration curve data

Parameters	ATOR	CLOP
Range	1-5µg/ml	5-120 µg/ml
Slope	1263645	2320480
SD of slope	0.274	0.143
Intercept	39606	15021
SD of Intercept	1443.16	2584.18
Correlation coefficient	0.9991	0.9992

Precision

Instrument precision was determined by performing injection repeatability test and the RSD values for ATOR and CLOP were found to be 0.2% and 0.1%, respectively. The intra-day and inter-day precision studies were carried out and the low RSD values indicate that the method is precise.

Accuracy

The accuracy of the method was determined by calculating recoveries of ATOR and CLOP by Method of standard addition. The recoveries were found to be 98.7 – 100.17% and 99.6 - 100.2%

for ATOR and CLOP, respectively (Table 2). The high values indicate that the method is accurate.

The regression analysis of Recovery data are shown in Table 2.

Table 2: Recovery data

Level of % recovery	Mean %recovery		%RSD	
	ATOR	**CLOP**	**ATOR**	**CLOP**
50	99.99	100.14	0.394	0.192
100	99.99	100.26	0.959	0.125
150	99.99	100.09	0.127	0.120

Limit of detection and limit of quantification

The LOD and LOQ were measured by using an equation. The detection limits for ATOR and CLOP were 1.388 µg/ml and 4.164 µg/ml, respectively, while quantitation limits were 8.56µg/ml and 25.68 µg/ml respectively. The above data shows that a micro gram quantity of both drugs can be accurately and precisely determined.

Stability of standard and sample solutions

Stability of standard and sample solution of ATOR and CLOP were evaluated at room temperature for 48 h. The relative standard deviation was found to be below 2.0%. It showed that both standard and sample solution were stable up to 48 h at room temperature.

Specificity

The specificity study was carried out to check the interference from the excipients used in the formulations by preparing synthetic mixture containing both the drugs and excipients. The chromatogram showed peaks for both the drugs without any interfering peak and the recoveries of both the drugs were above 98%.

Table 3: Summary of Validation Results

S.No	Parameters	Conditions	ATOR	CLOP	Acceptance criteria
1	System suitability	Tailing factor	1.47	1.6	**< 2**
		Theoretical plates	5415	5695	**> 2000**
2	Linearity	Correlation Coefficient (r^2)	0.9991	0.9992	**0.999**
3	Accuracy (% RSD)	50%	0.394	0.192	**< 2**
		100%	**0.959**	0.125	
		150%	**0.127**	0.120	
4	Precision (% RSD)	system	1.510	0.510	**< 2**
		Method	0.275	0.141	

5	Specificity	Interference	No	No	**No**
6	Robustness	Flow rate 0.9ml/min	0.619	0.241	**< 2**
		Flow rate 1.1 ml/min	0.96	0.306	
		Mobile phase 40:60 v/v	0.259	0.259	
		Mobile phase 20:80 v/v	0.219	0.4	
7	Ruggedness (%RSD)	Analyst 1	0.528	0.39	< 2
		Analyst 2	0.802	0.274	

Analysis of marketed formulations

An accurately weighed quantity of capsule powder equivalent to 75.0 mg of CLP and 10.0 mg of ATR was transferred to 100 mL volumetric flask, sonicated for 30 minutes with sufficient quantity of methanol and volume was made up to mark with methanol. The contents of the flask were filtered through Whatmann filter paper (no.41). A 5.0 mL portion of the filtrate was further diluted to 50.0 mL with a water and ACN (70:30)v/v. The sample solution was injected and the chromatogram was recorded. The content of CLOP and ATOR were calculated by comparison of the standard area and sample area. The quantification was carried out by keeping these values to the straight line equation of calibration curve.

Table: 4. Analysis of marketed formulation

Tablet	**Label claimed(mg)**		**Conc.found (mg)**		**% Recovery**	
Atorfit-CV	ATOR	CLOP	ATOR	CLOP	ATOR	CLOP
Capsules	10	75	10	75	99.80	100.0

CONCLUSION

This developed and validated method for simultaneous analysis of Atorvastatin and Clopidogrel in pharmaceutical preparations is very simple, rapid, accurate and precise. The method was successfully applied for determination of ATOR and CLOP in its pharmaceutical formulations. Moreover, it has advantages of short run time and the possibility of analysis of a large number of samples, both of which significantly reduce the analysis time per sample. Hence, this method can be conveniently used for routine quality control analysis of ATOR and CLOP in their pharmaceutical formulations.

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