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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ATORVASTATIN AND CLOPIDOGREL IN TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT

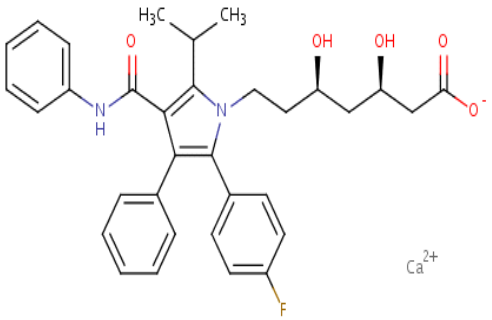
A new, simple, accurate and precise reversed phase high performance liquid chromatographic method was developed for the simultaneous quantitative estimation of Atorvastatin Calcium (ATOR) and Clopidogrel Bisulphate (CLOP) in combined capsule dosage form. The method was based on reversed phase liquid chromatography and separation was achieved on a Std kromasil (150 x 4.6 mm, 5). The Eluent was monitored by measuring the absorbance at wavelength 240 nm using a mixture of methanol and 0.01N Sodium dihydrogen ortho phosphate in the ratio of 50:50 (v/v) at pH 4 with a flow rate of 1.0 mL min^{-1}. The Calibration curves were found to be linear in the concentration range of 5-30 µg mL^{-1} and 37.5-225 µg mL^{-1} for ATOR and CLOP respectively. The proposed method was validated by testing for its linearity, recovery, repeatability and it was successfully employed for the rapid, specific and sensitive simultaneous quantitative estimation of Atorvastatin Calcium and Clopidogrel Bisulphate in capsules.

KEYWORDS: Atorvastatin, Calcium, Clopidogrel Bisulphate, RP-HPLC, Validation.

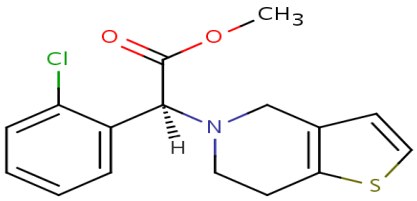
INTRODUCTION

Atorvastatin selectively and competitively inhibits the hepatic enzyme HMG-CoA reductase. HMG CoA reductase inhibitors (ATR) competitively inhibit the activity of HMG CoA reductase, the rate-limiting enzyme in cholesterol synthesis.[1] Inhibition of this enzyme results in a transient, modest decrease in cellular cholesterol concentration. The decrease in cholesterol concentration activates a cellular signaling cascade culminating in the activation of sterol regulatory element binding protein (SREBP), a transcription factor that up-regulates expression of the gene encoding the LDL receptor.[2] Increased LDL receptor expression causes increased uptake of plasma LDL, and consequently decreases plasma LDL-cholesterol concentration. Approximately 70% of LDL receptors are expressed by hepatocytes, with the remainder expressed by a variety of cell types in the body.[3]

Clopidogrel is an inhibitor of platelet aggregation; doses of 75 mg per day inhibit platelet aggregation by 40 to 60% at steady state, which occurs within 3 to 7 days.[4] Clopidogrel inhibits adenosine diphosphate (ADP) binding to its platelet receptor and subsequent ADP-mediated activation of the glycoprotein GPIIb/IIIa complex, thus inhibiting platelet aggregation [5]. Because clopidogrel irreversibly modifies the ADP receptor, platelets are affected for the remainder of their lifespan[6]. An active metabolite, not yet isolated, is responsible for the medication's activity. Platelet aggregation induced by agonists other than ADP is also inhibited by blocking the amplification of platelet activation by released ADP[7]. Clopidogrel does not inhibit phosphodiesterase activity.[8]



Structure of Atorvastatin



Structure of Clopidogrel

Atorvastatin calcium alone has been determined by Spectrophotometric methods like Stability indicating HPTLC method and RP-.HPLC.[9] Clopidogrel was also estimated using UV- method, Derivative Spectroscopy, HPLC, HPTLC and LCMS/MS. To the best of knowledge, only one method has been developed for the simultaneous determination of both the drugs in tablets include Q ratio and first method for simultaneous estimation of Atorvastatin Calcium and Clopidogrel from the derivative methods.[10] The present research work describes the rapid, accurate, sensitive and reproducible spectroscopic capsule formulation.

MATERIALS AND METHODS

Experimental Instrumentation

The HPLC system consisted of a LC Waters (Waters, Milford, MA, USA) using a Water's C18 Standard kromasil 150 x 4.6 mm, 5 column [11]. The system was equipped with a photodiode array detector (Water, 2996 model) and auto sampler (Waters, model 717 plus).

Data was processed using Empower Pro software (Waters, Milford, MA, USA). The mobile phase was pumped at a flow rate of 1.0 mL min−1. RT's were observed for Atorvastatin 2.662 min and Clopidogrel 4.252min respectively.

CHEMICALS AND REAGENTS

Reference standards of Atorvastatin Calcium and Clopidogrel were kindly supplied by Dr. Macs Bio pharma Pvt.ltd, Hyderabad, India with purity of 98.5% and 99.9% respectively.

Capsule formulation containing 10 mg of ATOR and 75 mg of CLOP was procured & manufactured by ZYDUS

CADILA COMPANY

Methanol Sodium di hydrogen orthophosphate, Potassium di hydrogen ortho-phosphate, Ortho-phosphroic acid, Acetonitrile, Milli Q water are the analytical grade chemicals used.

Method Development and Optimization of Chromatographic conditions

Selection of chromatographic condition: The drugs selected in the present study are Polar in nature and hence RP-HPLC method may be used. HPLC was selected for the separation because of its simplicity and suitability.[12]

Selection of wavelength (λ_{max})

In setting up the conditions for the development of the assay method the choice of detection wavelength was based on the PDA detector [13]. So in this method the peaks shapes of Atorvastatin and Clopidogrel were good at 240 nm.

Mobile phase: As the drugs are basic, an acidic mobile phase has been chosen. After a number of trails, the mobile phase was optimized with Phosphate buffer: methanol in proportion of 50:50 v/v respectively.

METHOD DEVELOPMENT

Chromatographic conditions:

Flow rate	**:**	**1** ml/min
Column	**:**	Kromasil C_{18} 150 x 4.6 mm, 5.
Detector wave length	**:**	240 nm
Column temperature	**:**	30 °C
Injection volume	**:**	10 L
Run time	**:**	7 min
Diluent	**:**	water and Methanol (50:50).

Preparation of diluent

Water and methanol was used in the ratio (1:1) for the preparation of all the sample solutions.

Working standards

Transferred about 5.0 mg of Atorvastatin working as standard into a 25 ml volumetric flask. And dissolved, diluted to 25 ml with diluent solution (Atorvastatin concentration-0.2 mg/ml).

Transferred about 15 mg of Clopidogrel working as standard into a 10 ml volumetric flask. And dissolved, diluted to 10 ml with diluent solution (Clopidogrel concentration-1.5 mg/ml).

Transferred 1 ml each of solution (Atorvastatin) and solution (Clopidogrel) into a 10 ml volumetric flask and made up to 10 ml with diluent. (Concentrations- 0.02 mg/ml, 0.15 mg/ml).

Analytical method-Optimization

The present study is to develop a new reverse phase liquid chromatographic method for simultaneous determination of Atorvastatin and Clopidogrel Capsule dosage form.

Table No: 5 Table showing Optimized Chromatographic Parameters

OPTIMIZED CHROMATOGRAPHIC CONDITIONS	
Mode of separation	isocratic elution
Mobile phase	Solvent-A: 0.01N NaH_2Po_4buffer pH-4 Solvent-B: methanol
Column	Std kromasil ,C18, 150 x 4.6 mm, 5.
Flow rate	1 mL/ min
Detection Wavelength	240 nm
Injection volume	10 l
Column oven temperature	Ambient (30 °C)
Run time	7 min

Quantitative determination of the drug by using the developed method
Sample: Atorvastatin and Clopidogrel

Label claim: 10 mg Atorvastatin + 75 mg of Clopidogrel
Assay procedure
The amount of drug was calculated by using the following formula

% Assay = At/As × Ws/Ds × Dt/Wt ×P/100 ×avg.weight/Label claim × 100
Where,
At = average area counts of sample preparation
As = average area counts of standard preparation
Ws = Weight of working standard taken in mg
Wt = Weight of sample taken in mg.
Dt = Sample dilution
Ds = Standard dilution
P = Percentage purity of working standard.

Acceptance criteria: The limit of assay is in between the 98 % - 102 %

VALIDATION
Typical validation characteristics which should be considered are listed below.
- Linearity & Range
- Accuracy
- Precision
- Ruggedness

After method development, the validation of the current method has been performed in accordance with USP requirements for assay determination which include accuracy, precision, selectivity, linearity, range, robustness and ruggedness.[14]

Accuracy
Formula

$$\% \text{ recovery} = \frac{\text{(Amount recovered)}}{\text{(Actual amount added)}} \times 100$$

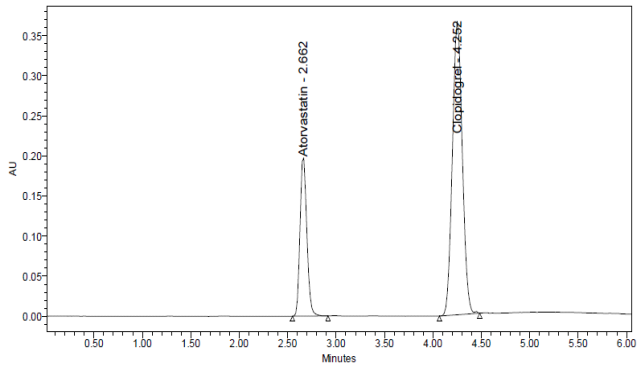
Acceptance criteria
Percentage recovery in all the cases should be between 98.0 and 102.0 %.

Precision:
- **System Precision**
- **Method Precision**
- **Intermediate Precision**

% RSD Formula**: (σ /μ) × 100**

Specificity
Blank, placebo solution, standard and sample solutions were injected into the HPLC system. Atorvastatin and Clopidogrel were homogenous and there was no interference at the retention time of Atorvastatin and Clopidogrel.[15]

RESULTS
OPTIMIZED METHOD

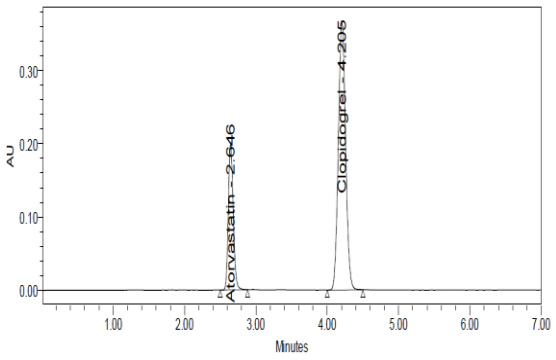


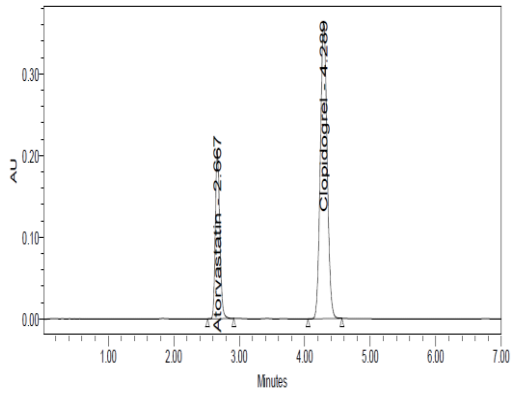
Peakresults

	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing	USP Resolution
1	Atorvastatin	2.662	971770	27.16	6924	1.12	
2	Clopidogrel	4.252	2606203	72.84	7879	1.05	9.74

Peak shape was good. The peaks are sharply resolved with less tailing and hence the trial-6 method is optimized for analysis. RT's were observed at Atorvastatin (2.662) and Clopidogrel (4.252).

ASSAY RESULTS



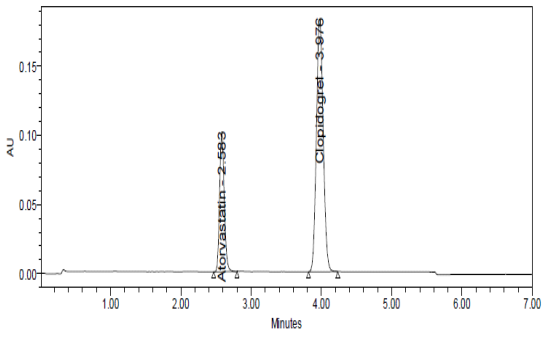


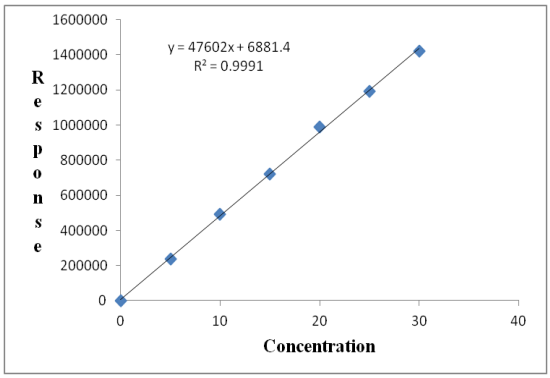
Standard chromatogram Sample

Chromatogram

S. No	Component name	Assay value
1.	Atorvastatin	100.45%
2.	Clopidogrel	99.88%

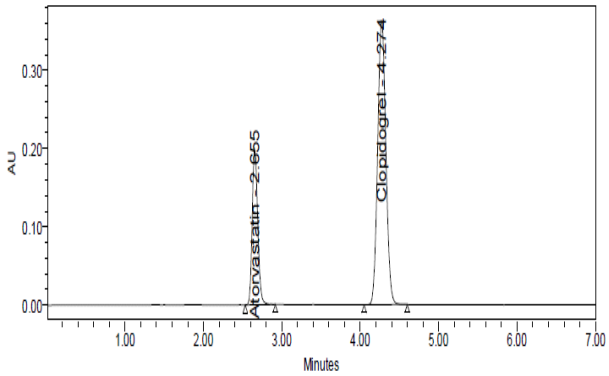
Validation





Accuracy4Calibration curve

Ruggedness



PEAK RESULTS

Peak Name: Atorvastatin

	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	Atorvastatin	2.653	976072	6993	1.16
2	Atorvastatin	2.655	974755	6803	1.16
3	Atorvastatin	2.655	977378	6759	1.17
4	Atorvastatin	2.658	975051	6975	1.15
5	Atorvastatin	2.661	975331	7277	1.12
6	Atorvastatin	2.667	974488	7025	1.18
Mean			975513		
Std. Dev.			1064.3		
% RSD			0.1		

Peak Name: Clopidogrel

	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	Clopidogrel	4.265	2638823	7914	1.05
2	Clopidogrel	4.271	2638224	7863	1.06
3	Clopidogrel	4.274	2636469	7931	1.06
4	Clopidogrel	4.276	2642214	7837	1.05
5	Clopidogrel	4.284	2638359	8010	1.06
6	Clopidogrel	4.289	2636754	7953	1.06
Mean			2638474		
Std. Dev.			2057.4		
% RSD			0.1		

SUMMARY AND DISCUSSION

Analytical method development and Validation has been developed for the determination of assay of Atorvastatin and Clopidogrel dosage form was performed for the parameters including-specificity, linearity and range, precision (system precision, method precision), intermediate precision (ruggedness), accuracy and Robustness. The summary of results obtained is appended below.

Total results for validation parameters

Parameter	Acceptance criteria	Results	
		Atorvastatin	Clopidogrel
Linearity and Range	Correlation coefficient should not be less than 0.999 over working range	Correlation coefficient = 0.9991	Correlation coefficient = 0.9997
Accuracy	Recovery at each level and % mean recovery should be between 100% to 150% with % RSD should not be more than 2.0%	Mean% recovery =99.2-99.1% %RSD=1.0-0.3	Mean% recovery =99.57-99.9 %RSD=0.4-0.7
Precision (Repeatability) system & method precision	% RSD should not be more than 2.0%	System precision SD=1167.0 %RSD=0.1	System precision SD=1675.1 %RSD= 0.1
		Method precision SD=6732.1 %RSD=0.7	Method precision SD=11020.6 %RSD=0.4
Specificity	There shouldn't be interference from blank and main peak. (Active)	There is no interference from blank and sample peak	There is no interference from blank and sample peak

The present work was developed and validated a HPLC method with PDA detector for the assay of Atorvastatin and Clopidogrel capsules to be employed in routine and stability tests [16]. In the method development of Atorvastatin and Clopidogrel project work carried out by incorporating the Reverse phase High Performance Liquid Chromatography (HPLC). Finally the method was developed & validated according to ICH guidelines for its various parameters.

CONCLUSION

The method was validated for system suitability, linearity, precision, accuracy, specificity, ruggedness, robustness and stability in analytical solution. The system suitability parameters were within limit, hence it was concluded that the system was suitable to perform the assay. The method shows linearity between the concentration ranges of 5-30 µg/ml for Atorvastatin 37.5-225 µg/ml for Clopidogrel. The %recovery of Atorvastatin and Clopidogrel was found to be in the range of about 99.2%, 99.57% and 99.85% respectively. As there was no interference due to excipients and mobile phase and good resolution found between peaks of Atorvastatin and Clopidogrel, the method was found to be specific. The method was robust and rugged as observed from insignificant variation in the results of analysis by changes in Flow rate, organic composition in mobile phase. Good agreement was seen in the assay results of pharmaceutical formulation by developed method & it can be concluded that the proposed method was a good approach for obtaining reliable results and found to be suitable for the routine analysis of Atorvastatin and Clopidogrel in Bulk & Pharmaceutical formulations.

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