ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Development and Validation of Analytical and Method for the Simultaneous Estimation of Clopidogrel Bisulphate and Atorvastatin Calcium in Bulk and in Tablet

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ABSTRACT

A simple, specific, accurate, precise and reproducible method has been developed and validated for the simultaneous estimation of Clopidogrel Bisulphate and Atorvastatin calcium in combined dosage form by RP-HPLC method. RP-HPLC estimation of drugs in selected combination was done using RP-Princeton SPHERE -100 C_{18} Column(4.6×250 mm, 5 µm) and ACN : Water (65:35) as mobile phase which shows sharp and resolved peak when detected at 227nm. The linearity range was found to be in concentration range 5-15 µg/mL for Clopidogrel Bisulphate (CLP) and 20-60 µg/mL for Atorvastatin calcium (ATR). The retention time for of Clopidogrel Bisulphate and Atorvastatin calcium were 4.3 and 9.4 respectively. The correlation coefficient was found to be 0.9987 (for CLP) and 0.9981 (for ATR). The mean percentage recovery was found to be 100.24 and 99.75 for of Clopidogrel Bisulphate and Atorvastatin calcium respectively. The % estimation of the drugs was found near to 100 % representing the accuracy of the method. Validation of the proposed method was carried out for its accuracy, precision, specificity and ruggedness according to ICH guidelines. The proposed method can be successfully applied in routine work for the determination of Clopidogrel Bisulphate and Atorvastatin calcium in combined dosage form.

Keywords: Clopidogrel Bisulphate , Atorvastatin calcium, RP-HPLC method, tablets.

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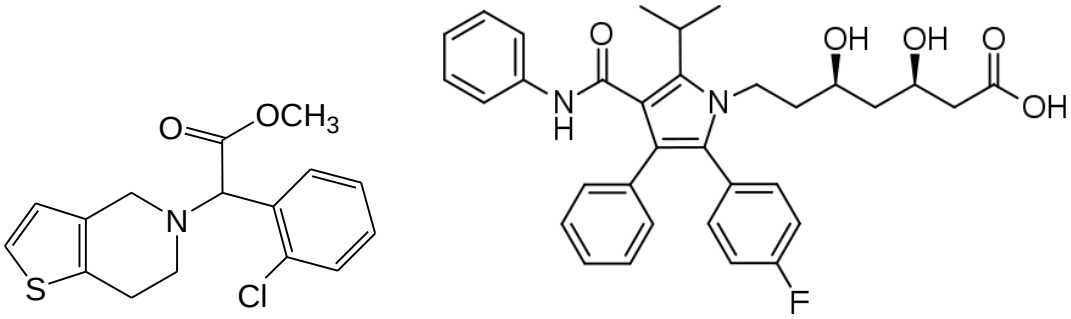
ISSN: 0975-8585

INTRODUCTION

Clopidogrel(CLP) is Antiplatelet Agents, Fibrinolytic Agents and Platelet Aggregation Inhibitors. The active metabolite of clopidogrel prevents binding of adenosine diphosphate (ADP) to its platelet receptor, impairing the ADP-mediated activation of the glycoprotein GPIIb/IIIa complex. It is proposed that the inhibition involves a defect in the mobilization from the storage sites of the platelet granules to the outer membrane. No direct interference occurs with the GPIIb/IIIa receptor.



CLP chemically is methyl(2S)-2-(2-chlorophenyl)-2-(6,7-dihydro-4H-thieno[3,2-c]pyridin-5-yl)acetate. Atorvastatin Calcium(ATR) is Anti-hyperlipidemic ATR chemically is (3R,5R)-7-[2-(4-flurophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]-3-5dihydroxyheptanoic acid. Literature survey revealed that the methods reported earlier were only for the analysis of single drug like UV-spectrophotometry[2] In combination methods are reported for ATR with Fenofibrate [1], Amlodipine [4], Telmisartan[3], Ezetimibe[6] CLP with Aspirin[9]. This paper presents simple, accurate, reproducible, rapid HPLC method for simultaneous analysis of the two components in tablet formulation.

MATERIALS AND METHODS

Instrument

High Performance Liquid Chromatography was performed with Systronic LC8600. Clopidogrel Bisulphate and Atorvastatin calcium were separated on a reverse-phase (250 mm × 4.6 mm) 5µm, C18 column RP-Princeton SPHER-100 column. Mobile phase filtration was performed by vaccum pump using 0.45 µm filter paper and degassed using Equitron Ultrasonicator.

Materials

Multi-component tablet Atorfit CV(ATR 20mg and CLP 75mg) manufactured by Ajanta Pharma ltd,Dist- Mumbai, Maharashtra. All chemicals and reagents used were of HPLC grade.

Mix Standard solution

Mix Stock solution containing CLP and ATR was prepared in methanol having concentration 1000 µg/ mL CLP and 4000 µg/ mL ATR. Aliquot of the standard solution was appropriately diluted with the mobile phase containing ACN : Water (65:35) to get the concentration of 10µg/mL for CLP and 40µg/mL for ATR respectively.

Procedure

The optimized chromatographic condition mentioned below was kept constant throughout the experimentation and mobile phase was allowed to equilibrate with stationary phase which was indicated by a steady line.

Column - RP-Princeton SPHERE -100 C_{18} Column(4.6×250 mm, 5 µm)
Detection Wavelength - 227 nm
Flow rate - 1.0 mL/min
Temperature: 25°C

A 20 µL solution of above mix standard was injected through manual injector and chromatogram was recorded using mobile phase containing Acetonitrile : Water (65:35) Clopidogrel Bisulphate and Atorvastatin calcium were resolved properly with sharp peak and showing reasonable retention time in the above selected mobile phase. A chromatogram for both drugs so recorded in shown in fig No.1and2.

Study of system suitability parameters

After equilibration of column with mobile phase, seven replicate injections of 20 µL solution of mix standard solution was injected through the manual injector and the chromatograms were recorded and the system suitability parameter were noted and values are shown in Table 1.

Study of Linearity Range

From the Aliquots of mixed standard stock solution volume was made up to mark with mobile phase to obtain CLP in the range of 5 to 15 µg/mL and ATR in the range of 20 to 60 µg/ml. respectively. The graphs of concentration of drug vs. area under curve were plotted for both the drugs. The correlation coefficient was found to be 0.9987 (for CLP) and 0.9981 (for ATR).

Assay in Marketed Formulation

An accurately weighed quantity of tablet powder equivalent to 40.0 mg of CLP and 10.0 mg of ATR was transferred to 25.0 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

ISSN: 0975-8585

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 mobile phase. The sample solution was injected and the chromatogram was recorded. The content of CLP and ATR were calculated by comparison of the standard area and sample area and results are shown in Table No2.

Validation

Accuracy: To ensure the reliability and accuracy of the method recovery studies were carried out by standard addition method. A known quantity of pure drug was added to pre-analysed sample and contents were reanalysed by proposed method and the mean % recovery were found to be 100.24 and 99.75 for CLP and ATR respectively.

Precision and Intermediate precision (Intraday and Inter day) shows the % Label claim values within limits (% R.S.D. not more than 2). The method was found to be précised. The ruggedness studies were carried out using different analyst variation. The results of intermediate precision and ruggedness results are shown in table No.3.

Robustness: Robustness of the method was investigated under a variety of conditions including changes of flow rate, percentage of Acetonitrile in the mobile phase, column oven temperature, and change in wavelength of measurement. The mixed standard solution is injected in five replicates and sample solution of 100% concentration is prepared and injected in triplicate for every condition and % R.S.D. of assay was calculated for each condition. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust results are shown in table No.4.

Linearity and Range: Linearity was determined for CLP in the range of 5-15 µg/mL; and for ATR, 20-60 µg/mL. The correlation coefficient ('r') values for both the drugs were >0.999.

RESULTS AND DISCUSSION

Table No.1 System Suitability Parameter

System Suitability Parameters	Proposed Method	
	CLP	ATR
Retention Time (t_R)	4.3	9.4
Capacity Factor (k')	1.19	1.00
Theoretical Plates (N)	2444	2316
Tailing Factor (T)	1.24	1.02
Resolution Factor (R)	8.14	--

ISSN: 0975-8585

Table No.2 Results of Marketed formulations and Recovery study

% Level	Mean Recovery	
	CLP	ATR
50	101.21	100.21
100	99.98	99.34
150	99.53	99.72
Mean ± SD	**100.24±0.68**	**99.75±0.82**
%R.S.D.	**0.732**	**0.327**

Table No.3 Intermediate precision and Ruggedness study

Parameter	% Assay	
	CLP	ATR
Ruggedness	**99.7**	98.61
Method Precision	**102.50**	99.43
Mean	**101.10**	99.02
SD	**1.98**	0.57982
% RSD	**1.96**	0.58556

Table No. 4 Robustness Study

Factor	Level	CLP		ATR	
		Mean of % Assay	% RSD	Mean of % Assay	% RSD
Flow Rate (mL/min)	0.8	99.81	0.021	99.09	1.070
	1.2	99.56	0.333	98.88	0.779
Percentage of ACN	55	99.50	0.419	98.77	0.622
	75	99.20	0.841	98.22	0.165
Column oven temperature(◦C)	20	100.02	0.325	99.05	1.020
	30	98.96	1.193	97.92	0.599
Measurement wavelength (nm)	242	103.50	0.69	98.72	0.551
	252	102.83	0.46	98.18	0.230

ISSN: 0975-8585

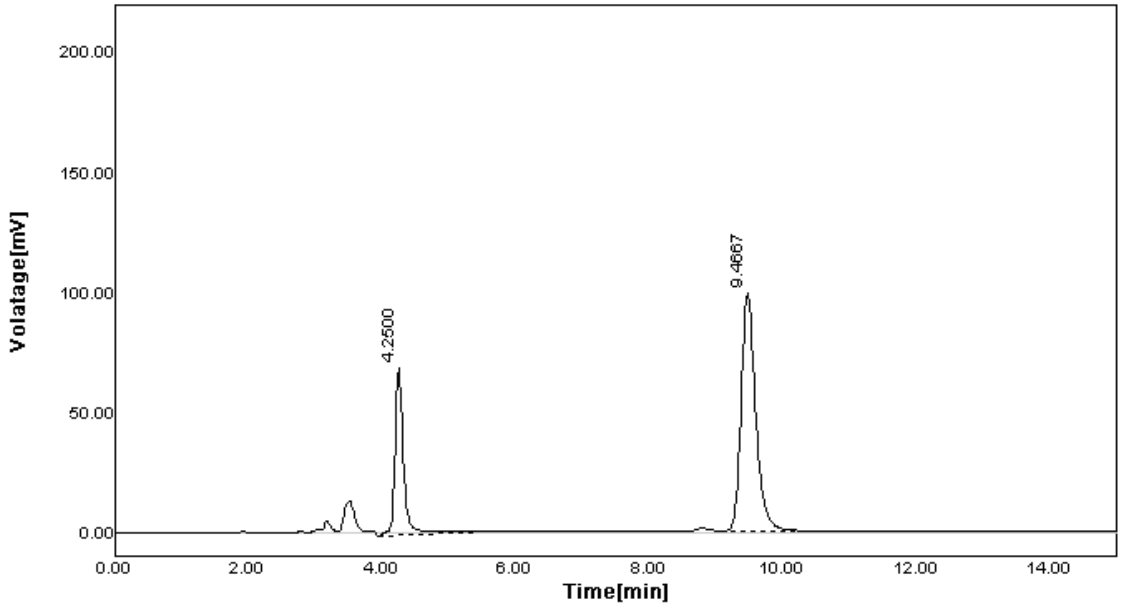


Fig No. 1: Standard Chromatogram

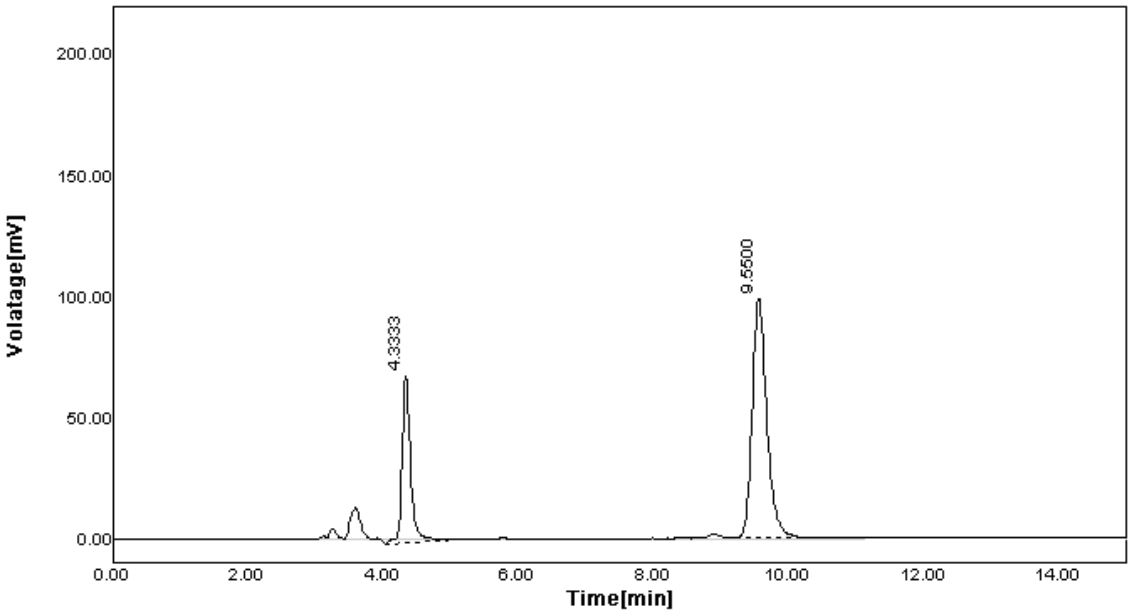


Fig No. 2: Sample Chromatogram

The optimised chromatographic conditions gave well resolved and sharp peaks of CLP and ATR with retention times 4.3 and 9.4 respectively. It was observed that the proposed method can be easily applied to marketed formulation and the statistical parameter viz. S.D., CV is in the acceptable range for quantitative determination of CLP and ATR. The method validation parameter like accuracy, precision, Linearity and range and specificity were found to be satisfactory.

ISSN: 0975-8585

CONCLUSION

The results obtained by the proposed method for determination of CLP and ATR are reliable, accurate and precise. The values of standard deviation were found satisfactory and the recovery studies were close to 100%. The method does not require prior separation of one drug from other. Hence it can be employed for routine quality control analysis of CLP and ATR in combined dosage form.

ACKNOWLEDGEMENTS

The authors are thankful to M.J. Bio Pharmaceutical Ltd. for providing standard drug samples and also to Hon'ble Loksevak Madhukarrao Chaudhari College of Pharmacy, Faizpur for providing the facilities to carry out the work.

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