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RESEARCH ARTICLE

Simultaneous RP-HPLC method for the estimation of Clopidogrel and Atorvastatin in bulk and its tablet dosage form

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ABSTRACT

The aim of present research work simple, precise, accurate and robust RP-HPLC method for the simultaneous estimation of Clopidogrel and Atorvastatin in bulk and tablet dosage form. The chromatographic separation was performed by Waters C_{18} (4.6 x 250mm, 5.0µm) column and the mobile phase was consisting of OPA buffer: Methanol in the ratio of 25:75. The flow rate was maintained at 1.0 ml/min and UV detection of wavelength was observed at 227 nm. The linearity was found to be 1µg/ml to 5µg/ml of Clopidogrel, 7.5µg/ml to 37.5µg/ml of Atorvastatin with correlation coefficient was found to be 0.999. The percentage RSD was found to be less than 2. All the validation parameters are stastically validated with according to ICH guidelines. So, the developed method was can be applied to the quality control analysis of analytical laboratories for the simultaneous estimation of clopidogrel and Atorvastatin.

Key words: Clopidogrel, Atorvastatin, RP-HPLC, Mobile phase, Validation

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CONTENTS

1. Introduction

Atorvastatin selectively and competitively inhibits the hepatic enzyme HMG-CoA reductase. As HMG-CoA reductase is responsible for converting HMG-CoA to mevalonate in the cholesterol biosynthesis pathway, this results in a subsequent decrease in hepatic cholesterol levels. Decreased hepatic cholesterol levels stimulates upregulation of hepatic LDL-C receptors which increases

hepatic uptake of LDL-C and reduces serum LDL-C concentrations.

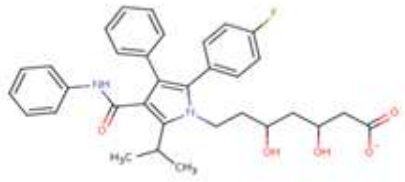

Fig 1: Chemical Structure of Atorvastatin

Clopidogrel binds to 5-HT type 1A serotonin receptors on presynaptic neurons in the dorsal raphe and on postsynaptic neurons in the hippocampus, thus inhibiting the firing rate of 5-HT-containing neurons in the dorsal raphe. Model drug also binds at dopamine type 2 (DA2) receptors, blocking presynaptic dopamine receptors. Model drug increases firing in the locus ceruleus, an area of brain where norepinephrine cell bodies are found in high concentration.

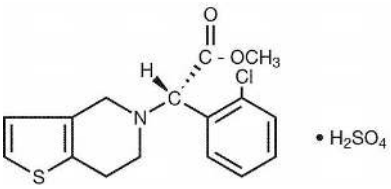

Fig 2: Structure of Clopidogrel

Atorvastatin and Clopidogrel are existing drugs. Literature reveals different methods for their analysis in their formulations. But our present plan is to develop a new, simple, precise& accurate method for its analysis in formulation after a detailed study a new RP-HPLC method was decided to be developed and validated.

2. Materials and Methods

Materials:

Table 1: Instruments used

S. No	Instrument	Model
1	HPLC	WATERS, software: Empower, 2695 separation module.2487 UV detector.
2	UV/VIS spectrophotometer	LABINDIA UV 3000^+
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

Table 2: Chemicals used

S.No	Chemical	Brand
1	Clopidogrel	Supplied by Pharmatrain
2	Atorvastatin	Supplied by Pharmatrain
3	Ortho phosphoric acid	FINAR chemical LTD
4	Water and Methanol for HPLC	Standard solutions Ltd
5	Acetonitrile for HPLC	Standard solutions Ltd
6	HCl, H_2O_2, NaOH	MERCK

HPLC Method Development:

Wave length selection:UV spectrum of 10 µg / ml Clopidogrel and Atorvastatin in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 227. At this wavelength both the drugs show good absorbance.

Optimization of Column:

Waters C_{18} (4.6 x 250mm, 5.0µm) was found to be ideal as it gave good peak shape and resolution at 1.2 ml/min flow.

Optimized Chromatographic Conditions:

Instrument used : High performance liquid chromatography equipped with Auto Sampler and UV detector
Temperature : Ambient
Column : Waters C_{18} (4.6 x 250mm, 5.0µm)
Buffer : 0.1% ortho phosphoric acid buffer
Mobile phase : 25% buffer: 75% Methanol
Flow rate : 1.0 ml per min
Wavelength : 227 nm
Injection volume : 20 µl
Run time : 10min.

Preparation of mobile phase:

Mix a mixture of above buffer 250 ml (25%) and 750 ml Methonol HPLC (75%) and degas in ultrasonic water bath for 5 minutes. Filter through 045 µ filter under vacuum filtration.

Standard Solution Preparation:

Accurately weigh and transfer 75mg of Clopidogrel & 10mg of Atorvastatin working standard into a 100ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)Further pipette 3ml of Clopidogrel & Atorvastatin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 75mg of Clopidogrel & 10mg Atorvastatin equivalent weight of the sample into a 100ml clean dry volumetric flask add about 70ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).Further pipette 3ml of Clopidogrel & Atorvastatin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure:

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for the Clopidogrel & Atorvastatin peaks and calculate the %Assay by using the formulae.

Method Validation

The developed method was validated by according to ICH guidelines Q_2(R_1). The validation Parameters like specificity, linearity, accuracy, precision, LOD & LOQ, robustness and system suitability.

3. Results and Discussion

System Suitability:

The system suitability parameters were determined by

preparing standard solutions of Clopidogrel and Atorvastatin and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2%.

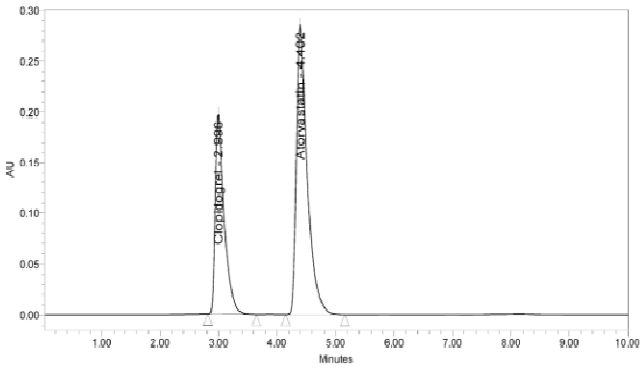

Fig 3: Chromatogram for system suitability

Acceptance Criteria: According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

Linearity:

Inject five serial dilutions into the chromatographic system and measure the peak area.The linearity range was found to lie from 1µg/ml to 5µg/ml of Clopidogrel, 7.5µg/ml to 37.5µg/ml 0f Atorvastatin. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results were shown in table 4.

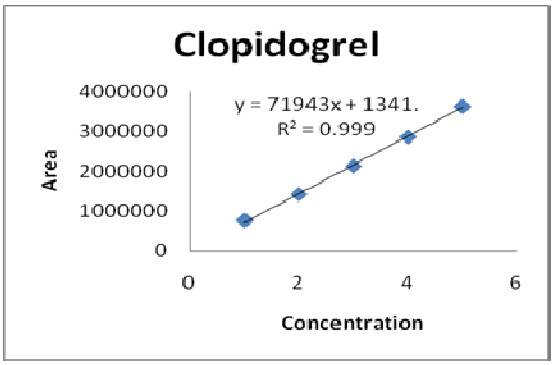

Fig 4: Calibration graph for Clopidogrel

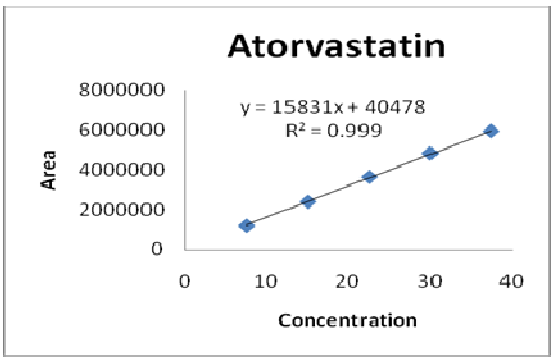

Fig 5: Calibration graph for Atorvastatin

Precision:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate Precision:

There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation.The standard solution was injected for five times and measured the area for all six injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Accuracy

For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same.Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Clopidogrel & Atorvastatin and calculate the individual recovery and mean recovery values.

LOD & LOQ:

LOD: The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

LOQ:

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

Robustness:

The standard and samples of Clopidogrel and Atorvastatin were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

4. Conclusion

The estimation of Clopidogrel and Atorvastatin was done by RP-HPLC. The linearity of Clopidogrel and Atorvastatin was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0%. The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 99.09% and 100.73% for Clopidogrel and Atorvastatin. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The LOD and LOQ for Clopidogrel was found to be 2.98 and 9.96 and LOD and LOQ for Atorvastatin was found to be 2.95 and 9.98. The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions. So, the developed method was simple, accurate, precise and robust. It can be suggest that routine quality control analysis of analytical laboratories.

Table 3: Results of system suitability parameters

S.No	Name	RT (min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Clopidogrel	2.996	2094603	196622	4.38	1.71	2947.68
2	Atorvastatin	4.402	3694090	286174		1.61	3826.77

Table 4: Area of different concentration of Clopidogrel and Atorvastatin

S. No	Clopidogrel		Atorvastatin	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	1	758140	7.5	1206413
2	2	1417589	15	2408658
3	3	2126451	22.5	3640611
4	4	2863952	30	4819318
5	5	3632151	37.5	5937929

Table 5: Results of Precision for Clopidogrel and Atorvastatin

Clopidogrel		Atorvastatin	
Injection	Area	Injection	Area
Injection-1	2101701	Injection-1	3705273
Injection-2	2105222	Injection-2	3710274
Injection-3	2107201	Injection-3	3715132
Injection-4	2115983	Injection-4	3725737
Injection-5	2117750	Injection-5	3728935
Average	2109571	Average	3717070
Standard Deviation	6972.8	Standard Deviation	10062.4
%RSD	0.33	%RSD	0.27

Table 6: Results of Intermediate Precision for Clopidogrel and Atorvastatin

Clopidogrel		Atorvastatin	
Injection	Area	Injection	Area
Injection-1	2117636	Injection-1	3732160
Injection-2	2126903	Injection-2	3745179
Injection-3	2130618	Injection-3	3747032
Injection-4	2131939	Injection-4	3751496
Injection-5	2140436	Injection-5	3757903
Injection -6	2146025	Injection -6	3764658
Average	2132260	Average	3749738
Standard Deviation	10016.8	Standard Deviation	11220.1
%RSD	0.47	%RSD	0.30

Table 7: Accuracy (recovery) data for Clopidogrel

%Concentration (at specification Level)	Area*	Amount Added(mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	1054643	37.5	37.75	99.92	99.09
100%	2079841	75	74.44	99.26	
150%	3083028	112.5	110.35	98.09	

*Average of three determinations

Table 8: Accuracy (recovery) data for Atorvastatin

%Concentration (at specification Level)	Area*	Amount Added(mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	1882082	5	5.09	101.87	100.73
100%	3676645	10	9.95	99.51	
150%	5588103	15	15.12	100.82	

*Average of three determinations

Table 9: LOD & LOQ Results for Clopidogrel and Atorvastatin

Drug name	LOD	LOQ
Clopidogrel	2.98	2.95
Atorvastatin	9.96	9.98

Table 10: Results for variation in flow for Clopidogrel

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	2967.74	1.72
2	1.0	2947.68	1.71
3	1.1	2989.44	1.74

Table 11: Results for variation in flow for Atorvastatin

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	3758.67	1.67
2	1.0	3826.77	1.61
3	1.1	3763.48	1.71

* Results for actual flow (1.0ml/min) have been considered from Assay standard.

Table 12: Results for variation in mobile phase composition for Clopidogrel

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2071.65	1.78
2	*Actual	2947.68	1.71
3	10% more	2994.24	1.77

Table 13: Results for variation in mobile phase composition for Atorvastatin

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3224.22	1.68
2	*Actual	3826.77	1.61
3	10% more	3824.86	1.61

* Results for actual Mobile phase composition have been consideredfrom Accuracy standard.

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