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Research Article

Method Validation and Stability Evaluation for the Simultaneous Estimation of Clopidogrel and Atorvastatin in Tablet Dosage Forms by RP-HPLC

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Abstract

Objectives: The study aims to develop and validate a simple, accurate, precise, economical method for the simultaneous estimation of the Clopidogrel and Atorvastatin in tablet dosage form by RP-HPLC method.

Methods: The chromatogram was run through Standard Discovery C8 (250x4.6mm, 5m.) The Mobile phase containing Buffer 0.1% Potassium Dihydrogen Phosphate: Acetonitrile taken in the ratio 45:55 was pumped through column at a flow rate of 1 ml/min. pH was adjusted to 3.8 with dil. Orthophosphoric acid solution. Buffer used in this method was potassium dihydrogen phosphate solution. Temperature was maintained at 30°C. Optimized wavelength selected Clopidogrel and Atorvastatin was 247nm.

Results: Retention time of Clopidogrel and Atorvastatin were found to 2.555min and 3.224min. With the optimized chromatographic conditions, the drug was linear in the concentration range of 0 – 150 µg/ml. The correlation coefficient was found to be 0.999. The average percentage assay in formulation was found to be 99.60% and 99.14% for Clopidogrel and Atorvastatin respectively. %RSD of the Clopidogrel and Atorvastatin were and found to be 0.4 and 0.3 respectively. %Recovery was obtained as 99.13% and 99.40% for Clopidogrel and Atorvastatin respectively. LOD, LOQ values obtained from regression equations of Clopidogrel and Atorvastatin were 0.26, 0.79 and 0.04, 0.11 respectively. Regression equation of Clopidogrel is y = 16467x + 5787, and y =34860x+5859 of Atorvastatin.

Conclusion: Hence the suggested RP-HPLC method can be used for routine analysis of Clopidogrel and Atorvastatin in API and Pharmaceutical dosage form.

Keywords: Atorvastatin; Clopidogrel; RP-HPLC; Stability; Simultaneous Estimation; Validation

Introduction

Clopidogrel, an antiplatelet agent structurally and pharmacologically similar to ticlopidine, is used to inhibit blood clots in a variety of conditions such as peripheral vascular disease, coronary artery disease, and cerebrovascular disease. Clopidogrel is sold under the name Plavix by Sanofi and Bristol-Myers Squibb. The drug is an irreversible inhibitor of the P2Y12 adenosine diphosphate receptor found on the membranes of platelet cells. Clopidogrel use is associated with several serious adverse drug reactions such as severe neutropenia, various forms of hemorrhage, and cardiovascular edema. Chemically clopidogrel is methyl (2S)-2-(2-chlorophenyl)-2-{4H,5H,6H,7H-thieno[3,2-c]pyridin-5-yl}acetate (Figure 1).

Atorvastatin (Lipitor) is a member of the drug class known as statins. It is used for lowering cholesterol. Atorvastatin is a competitive inhibitor of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-determining enzyme in cholesterol biosynthesis via the mevalonate pathway. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate. Atorvastatin acts primarily in the liver. Decreased hepatic cholesterol levels increases hepatic uptake of cholesterol and reduces plasma cholesterol levels Chemically atorvastatin is known as (3R,5R)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-(propan-2-yl)-1H-pyrrol-1-yl]-3,5 dihydroxyheptanoic acid (Figure 2).

Many methods like UV, RP-HPLC have been developed but with high retention time and run time in this study an accurate, precise, economic, method was developed for the assay related substance for the simultaneous estimation of Atorvastatin and Clopidogrel in pharmaceutical dosage forms. RP-HPLC methods [1-5]. By UV

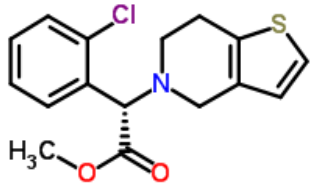

Figure 1: Structure of Clopidogrel.

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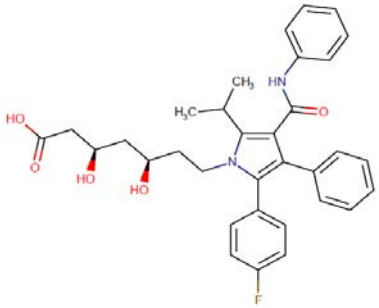

Figure 2: Structure of Atorvastatin.

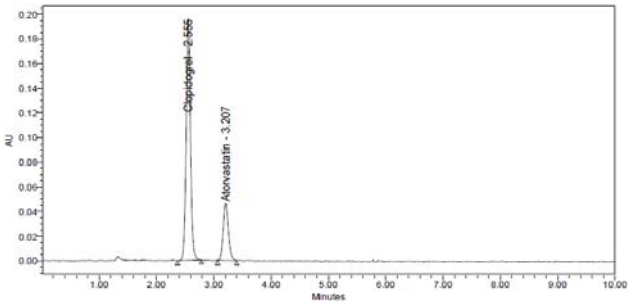

Figure 3: Optimized Chromatogram.

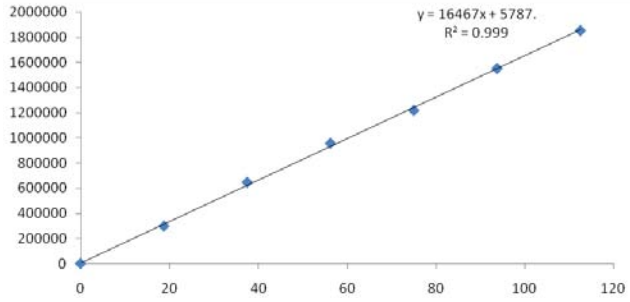

Figure 4: Calibration curve of Clopidogrel.

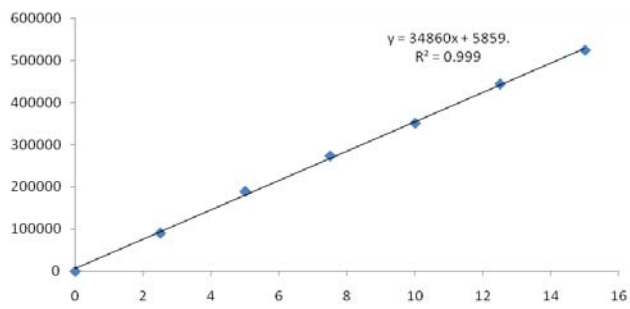

Figure 5: Calibration curve of Atorvastatin.

method [6] in bulk and combined dosage formulations [7-9]. In capsule dosage forms [10] in human plasma, Human Serum and Rat plasma [11-13]. This method seems to be economic with less retention time. Since the retention time was less the run time also decreased. The method developed can be applicable for quality control studies in industries.

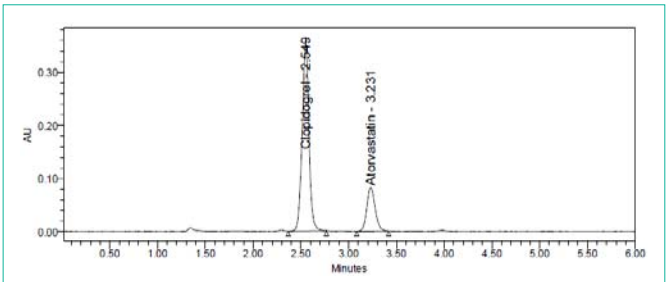

Figure 6: Accuracy 50% Chromatogram of Clopidogrel and Atorvastatin.

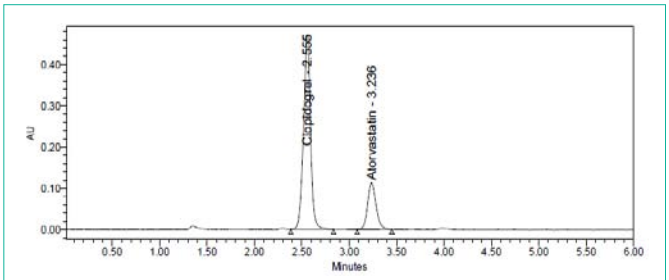

Figure 7: Accuracy 100% Chromatogram of Clopidogrel and Atorvastatin.

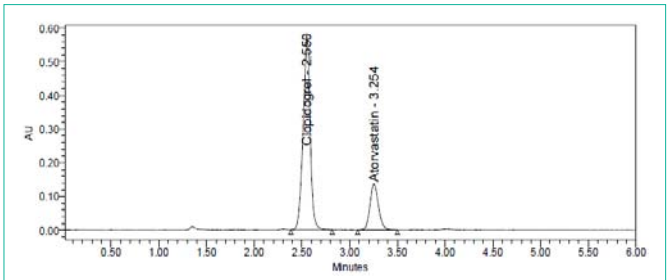

Figure 8: Accuracy 150% Chromatogram of Clopidogrel and Atorvastatin.

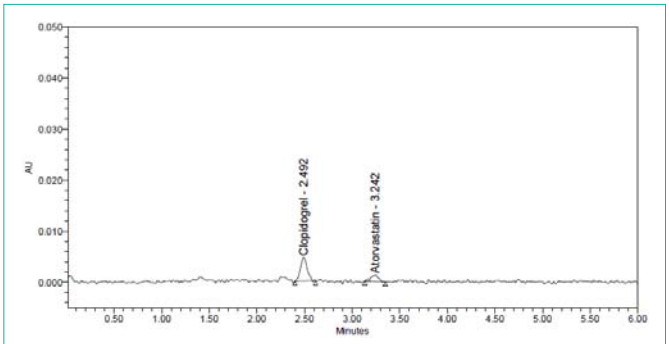

Figure 9: LOD Chromatogram of Standard.

Materials and Methods

Materials

Clopidogrel and Atorvastatin pure drugs (API), Combination Clopidogrel and Atorvastatin tablets (Axigrel AT), was obtained from Axis Life Science Pvt Ltd. Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dehydrogenate ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and reagents used were analytical grade and procured from Rankem Laboratories Pvt Ltd India.

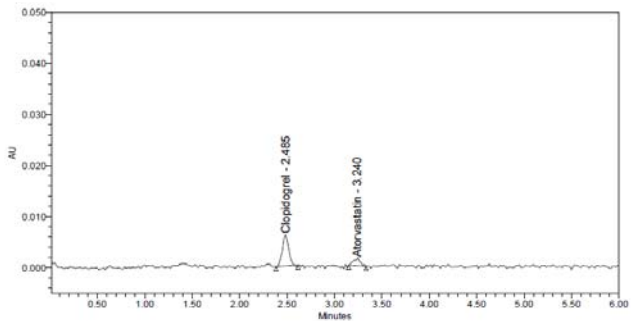

Figure 10: LOQ Chromatogram of Standard.

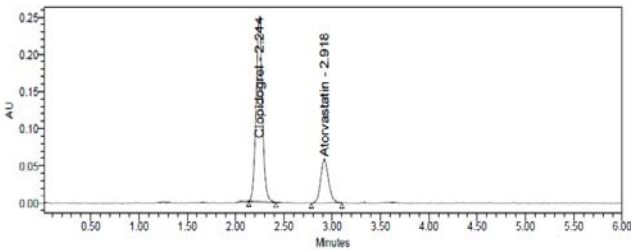

Figure 11: Flow plus Chromatogram of Clopidogrel and Atorvastatin.

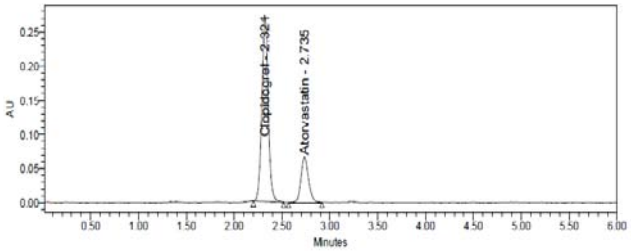

Figure 12: Mobile phase plus Chromatogram of Clopidogrel and Atorvastatin.

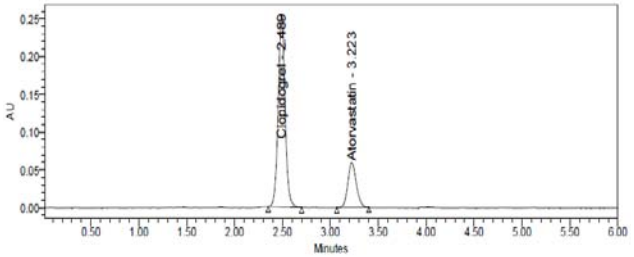

Figure 13: Temperature plus Chromatogram of Clopidogrel and Atorvastatin.

Instrumentation

Electronics Balance-Denver, p^H meter -BVK enterprises, India, Ultrasonicator-BVK enterprises, WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Clopidogrel and Atorvastatin solutions.

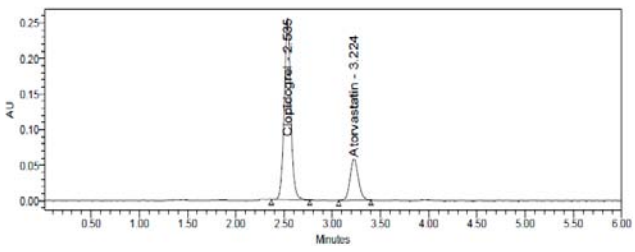

Figure 14: Chromatogram of working standard solution.

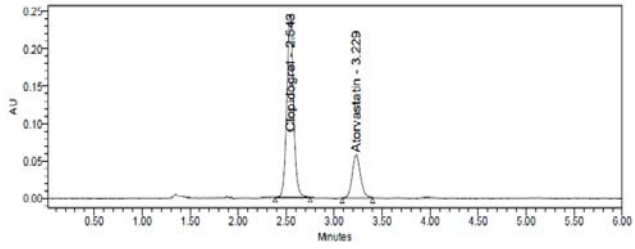

Figure 15: Chromatogram of working sample solution.

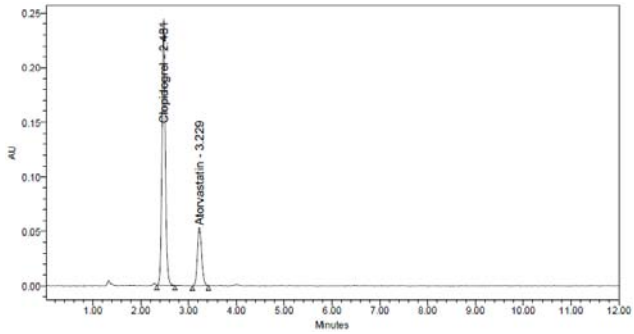

Figure 16: Acid chromatogram of Clopidogrel and Atorvastatin.

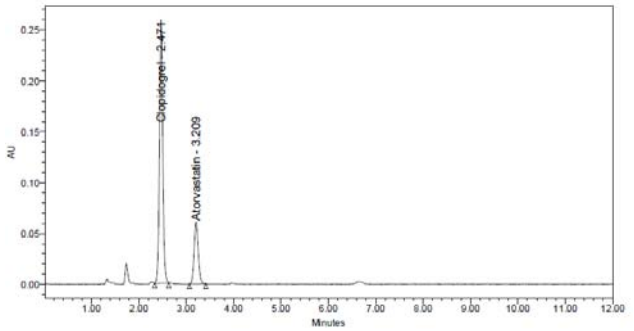

Figure 17: Base chromatogram of Clopidogrel and Atorvastatin.

Methods

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50.

Preparation of Standard stock solutions: Accurately weighed 18.75mg of Clopidogrel, 2.5mg of Atorvastatin and transferred to individual 25ml volumetric flasks separately. 3/4th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1

Table 1: Method development: Method development was done by changing various, mobile phase ratios, buffers etc.

Parameters	Trial 1	Trial 2	Trial 3	Trial 4	Optimized Method
Mobile phase	Water and Acetonitrile (50:50)	Water**:** Acetonitrile (50:50)	OPA: Acetonitrile (50:50)	**50**% OPA buffer: 50% Acetonitrile	55% 0.1% OPA buffer: 45% Acetonitrile
Flow rate	**1** ml/min	**1** ml/min	**1.2** ml/min	**1** ml/min	**1** ml/min
Column	BDS C18 (4.6 x 150mm, 5µm)	Waters C18 (4.6 x 150mm, 5µm)	Waters C18 (4.6 x 150mm, 5µm)	Discovery C8 (4.6 x 250mm, 5µm)	Altima C18 (4.6 x 150mm, 5µm)
Detector wave length	247nm	247nm	247nm	247nm	220nm
Column temperature	30°C	30°C	30°C	30°C	30°C
Injection volume	10mL	10mL	10mL	10mL	10mL
Run time	8.0 min	14 min	10 min	10 min	**6**min
Diluent	Water and Acetonitrile in the ratio 50:50	Water and Acetonitrile in the ratio 50:50	Water and Acetonitrile in the ratio 50:50	Water and Acetonitrile in the ratio 50:50	Water and Acetonitrile in the ratio 50:50

Table 2: Chromatographic conditions.

Trials	Results
Trial 1	Both were eluted but resolution was less & peak shape also not good so, further trial is carried out.
Trial 2	Both peaks were eluted but peak shapes were good but retention time was more than literature review So, Further Trial is carried out
Trial 3	Clopidogrel & Atorvastatin both peak are eluted but Retention was more so, further trail was carried.
Trial 4	Clopidogrel & Atorvastatin both peak are eluted but retention time was more so, further trial is Carried out
Optimized Method	Both peaks have good resolution, tailing factor, theoretical plate count and resolution.

Table 3: System suitability parameters for Clopidogrel and Atorvastatin.

S no	Clopidogrel			Atorvastatin			Resolution
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	
1	2.535	6881	1.07	3.221	6702	1.11	4.3
2	2.543	6609	1.03	3.223	6810	1.08	4.3
3	2.544	6620	1.03	3.224	6907	1.13	4.7
4	2.548	6826	1.05	3.224	6975	1.1	4.7
5	2.584	6830	1.04	3.233	6947	1.1	4.9
6	2.589	6484	1.05	3.244	6850	1.11	4.8

Table 4: Linearity data for Clopidogrel and Atorvastatin.

Clopidogrel		Atorvastatin	
Conc (µg/ml)	Peak area	Conc (µg/ml)	Peak area
0	0	0	0
18.75	296899	2.5	90425
37.5	646222	5	189018
56.25	956626	7.5	273091
75	1218095	10	351010
93.75	1552319	12.5	443823
112.5	1854090	15	523808

Table 5: System precision data of Clopidogrel and Atorvastatin**.**

S. No	Area of Clopidogrel	Area of Atorvastatin
1	1208220	352812
2	1203948	352344
3	1210969	356964
4	1224754	359020
5	1214415	353644
6	1204448	351917
Mean	1211126	354450
S.D	7760.5	2877.1
%RSD	0.6	0.8

and 2. (750µg/ml of Clopidogrel and 100µg/ml of Atorvastatin).

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (75µg/ml Clopidogrel of and 10µg/ml of Atorvastatin).

Preparation of Sample stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100ml volumetric flask, 50ml of diluents was added and sonicated for 25min, further the volume was made up with diluent and filtered by HPLC filters (750µg/ml of Clopidogrel and 100µg/ml of Atorvastatin).

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (75µg/ml of Clopidogrel and 10µg/ml of Atorvastatin).

Table 6: Repeatability data of Clopidogrel and Atorvastatin.

S. No	Area of Clopidogrel	Area of Atorvastatin
1	1208503	351049
2	1204001	352413
3	1207132	350097
4	1206232	352874
5	1201708	352019
6	1217089	352012
Mean	1207444	351744
S.D	5300.4	1006.7
%RSD	0.4	0.3

Table 7: Intermediate precision data of Clopidogrel and Atorvastatin.

S. No	Area of Clopidogrel	Area of Atorvastatin
1	1208783	351572
2	1210503	352049
3	1204001	352413
4	1207132	352874
5	1201708	350019
6	1207089	352012
Mean	1206536	351823
S.D	3199.5	985.6
%RSD	0.3	0.3

Table 8a: Accuracy data of Clopidogrel.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	37.5	37.207992	99.22	99.13%
	37.5	36.985183	98.63	
	37.5	37.425518	99.8	
100%	75	74.537074	99.38	
	75	73.998057	98.66	
	75	73.880428	98.51	
150%	112.5	110.82893	98.51	
	112.5	112.23417	99.76	
	112.5	112.11265	99.66	

Preparation of buffer 0.01N Kh2po4 Buffer: Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degtas to sonicate and finally make up the volume with water then added 1ml of Triethylamine then PH adjusted to 3.8 with dil. Orthophosphoric acid solution.

Results and Discussion

Clopidogrel and Atorvastatin were eluted at 2.555min and 3.207min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated (Figure 3).

System suitability

All the system suitability parameters were within the range and satisfactory as per ICH guidelines (Table 3) [14].

Table 8b: Accuracy data of Atorvastatin.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	5	4.9640562	99.28	99.40%
	5	4.9474182	98.95	
	5	4.9121916	98.24	
100%	10	9.9978199	99.98	
	10	9.9918531	99.92	
	10	9.911331	99.11	
150%	15	14.85066	99	
	15	14.998537	99.99	
	15	15.020683	100.14	

Table 9: Sensitivity table of Clopidogrel and Atorvastatin.

Molecule	LOD	LOQ
Clopidogrel	0.26	0.44
Atorvastatin	0.79	0.11

Table 10: Robustness data for Clopidogrel and Atorvastatin.

S.no	Condition	%RSD of Clopidogrel	%RSD of Atorvastatin
1	Flow rate (-) 0.9ml/min	0.7	1.4
2	Flow rate (+) 1.1ml/min	0.8	0.3
3	Mobile phase (-) 60B:40A	0.2	0.7
4	Mobile phase (+) 45B:45A	0.7	0.7
5	Temperature (-) 25°C	0.9	1.2
6	Temperature (+) 35°C	0.4	0.4

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

Six linear concentrations of Clopidogrel (18.75-112.5µg/ml) and Atorvastatin (2.5-15µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Clopidogrel was y =16467.x + 5787 and of Atorvastatin was y = 34860x + 5859 Correlation coefficient obtained was 0.999 for the two drugs (Table 4 Figure 4 & 5).

System Precision

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and %RSD were calculated for two drugs. %RSD obtained as 0.6% and 0.8% respectively for Clopidogrel and Atorvastatin. As the limit of Precision was less than "2" the system precision passed in this method and the results were within the limits (Table 5).

Repeatability

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained

Table 11: Assay Data of Clopidogrel & Atorvastatin.

S.no	Standard Area	Sample area	% Assay	S. no	Standard Area	Sample area	% Assay
1	1208220	1208503	99.68	1	352812	351049	98.94
2	1203948	1204001	99.31	2	352344	352413	99.33
3	1210969	1207132	99.57	3	356964	350097	98.67
4	1224754	1206232	99.5	4	359020	352874	99.46
5	1214415	1201708	99.12	5	353644	352019	99.21
6	1204448	1217089	100.39	6	351917	352012	99.21
Avg	1211126	1207444	99.6	Avg	354450	351744	99.14
Stdev	7760.5	5300.4	0.44	Stdev	2877.1	1006.7	0.2837
%RSD	0.6	0.4	0.44	%RSD	0.8	0.3	0.3

Table 12a: Degradation Data of Clopidogrel.

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.61	0.544	0.589
2	Alkali	2.93	0.461	0.529
3	Oxidation	1.81	0.523	0.592
4	Thermal	0.51	0.427	0.532
5	UV	0.62	0.321	0.421
6	Water	0.62	0.357	0.522

Table 12a: Degradation Data of Atorvastatin.

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.76	0.546	0.772
2	Alkali	2.95	0.6	0.733
3	Oxidation	1.91	0.578	0.635
4	Thermal	0.62	0.536	0.756
5	UV	0.98	0.632	0.698
6	Water	0.62	0.535	0.725

areas were mentioned in the above table. Average area, standard deviation and %RSD were calculated for two drugs and obtained as 0.4% and 0.3% respectively for Clopidogrel and Atorvastatin. As the limit of Precision was less than "2" the system precision passed in this method and the results were within the limits (Table 6).

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area, standard deviation and %RSD were calculated for two drugs and obtained as 0.3% and 0.3% respectively for Clopidogrel and Atorvastatin. As the limit of Precision was less than "2" the system precision passed in this method and the results were within the limits.

Intermediate precision (Day_ Day Precision)

Table 7.

Accuracy

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.13% and 99.40% for Clopidogrel and Atorvastatin respectively (Table 8, Figure 6-8).

Sensitivity

Table 9 Figure 9 & 10.

Robustness

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (60B:40A), mobile phase plus (55B:45A), temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit (Table 10, Figure 11-13).

Assay

Axis Life Science Pvt Ltd (Axigrel AT), bearing the label claim Clopidogrel 75mg, Atorvastatin 10mg. Assay was performed with the above formulation.. Average % Assay for Clopidogrel and Atorvastatin obtained was 99.60% and 99.14% respectively (Table 11 Figure 14 & 15).

Degradation

Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation (Table 12, Figure 16 & 17).

Conclusion

A new method was established for simultaneous estimation of Clopidogrel and Atorvastatin by RP-HPLC method. The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Clopidogrel and Atorvastatin in pharmaceutical dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines. The Sample recoveries in all formulations were in good agreement with their respective label claims. Hence the suggested RP-HPLC method can be used for routine analysis of Clopidogrel and Atorvastatin in API and Pharmaceutical dosage form.

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