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Research article ***RP-HPLC***

Determination of atorvastatin and clopidogrel by using RP-HPLC method in pure and its pharmaceutical dosage form

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ABSTRACT

A rapid and precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validation of Atorvastatin and Clopidogrel, in its pure form as well as in capsule dosage form. Chromatography was carried out on a Phenomenex Gemini C18 (4.6×250mm) 5µ column using a mixture of Methanol: TEA Buffer (65:35 v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 230nm. The retention time of the Atorvastatin and Clopidogrel was 2.121, 3.643 ±0.02min respectively. The method produce linear responses in the concentration range of 5-25mg/ml of Atorvastatin and 30-187.5mg/ml of Clopidogrel. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Atorvastatin, Clopidogrel, RP-HPLC, validation.

INTRODUCTION

Analytical chemistry[1]

Analytical chemistry is a scientific discipline used to study the chemical composition, structure and behaviour of matter. The purposes of chemical analysis are together and interpret chemical information that will be of value to society in a wide range of contexts. Quality control in manufacturing industries, the monitoring of clinical and environmental samples, the assaying of geological specimens, and the support of fundamental and applied research are the principal applications. Analytical chemistry involves the application of a range of techniques and methodologies to obtain and assess qualitative, quantitative and structural information on the nature of matter.

- ❖ Qualitative analysis is the identification of elements, species and/or compounds present in sample.
- ❖ Quantitative analysis is the determination of the absolute or relative amounts of elements, species or compounds present in sample.

Structural analysis is the determination of the spatial arrangement of atoms in an element or molecule or the identification of characteristic groups of atoms (functional groups). An element, species or compound that is the subject of analysis is known as analyte. The remainder of the material or sample of which the analyte(s) form(s) a part is known as the matrix.

The gathering and interpretation of qualitative, quantitative and structural information is essential to many aspects of human endeavour, both terrestrial and extra-terrestrials. The maintenance of an improvement in the quality of life throughout the world and the management of resources heavily on the information provided by chemical analysis. Manufacturing industries use analytical data to monitor the quality of raw materials, intermediates and finished products. Progress and research in many areas is dependent on establishing the chemical composition of man-made or natural materials, and the monitoring of toxic substances in the environment is of ever increasing importance. Studies of biological and other complex systems are supported by the collection of large amounts of analytical data. Analytical data are required in a wide range of disciplines and situations that include not just chemistry and most other sciences, from biology to zoology, butte arts, such as painting and sculpture, and archaeology. Space exploration and clinical diagnosis are

two quite desperate areas in which analytical data is vital. Important areas of application include the following.

Quality control

(**QC**) in many manufacturing industries, the chemical composition of raw materials, intermediates and finished products needs to be monitored to ensure satisfactory quality and consistency. Virtually all consumer products from automobiles to clothing, pharmaceuticals and foodstuffs, electrical goods, sports equipment and horticultural products rely, in part, on chemical analysis. The food, pharmaceutical and water industries in particular have stringent requirements backed by legislation for major components and permitted levels of impurities or contaminants. The electronic industry needs analyses at ultra-trace levels (parts per billion) in relation to the manufacture of semi-conductor materials. Automated, computer-controlled procedures for process-stream analysis are employed in some industries.

Monitoring and control of pollutants

The presence of toxic heavy metals (e.g., lead, cadmium and mercury), organic chemicals (e.g., polychlorinated biphenyls and detergents) and vehicle exhaust gases (oxides of carbon, nitrogen and sulphur, and hydrocarbons) in the environment are health hazards that need to be monitored by sensitive and accurate methods of analysis, and remedial action taken. Major sources of pollution are gaseous, solid and liquid wastes that are discharged or dumped from industrial sites, and vehicle exhaust gases.

Clinical and biological studies

The levels of important nutrients, including trace metals (e.g., sodium, potassium, calcium and zinc), naturally produced chemicals, such as cholesterol, sugars and urea, and administered drugs in the body fluids of patients undergoing hospital treatment require monitoring. Speed of analysis is often a crucial factor and automated procedures have been designed for such analyses.

Geological assays

The commercial value of ores and minerals are determined by the levels of particular metals, which must be accurately established. Highly accurate and reliable analytical procedures must be used for this purpose, and referee laboratories are sometimes employed where disputes arise.

Fundamental and applied research

The chemical composition and structure of materials used in or developed during research programs in numerous disciplines can be of significance. Where new drugs or materials with potential commercial value are synthesized, a complete chemical characterization maybe required involving considerable analytical work. Combinatorial chemistry is an approach used in pharmaceutical research that generates very large numbers of new compounds requiring confirmation of identity and structure.

Analytical techniques

There are numerous chemical or physico-chemical processes that can be used to provide analytical information. The processes are related to a wide range of atomic and molecular properties and phenomena that enable elements and compounds to be detected and/or quantitatively measured under controlled conditions. The underlying processes define the various *analytical techniques*. The more important of these are listed in Table.No.1 together with their suitability for qualitative, quantitative or structural analysis and the levels of analyte(s) in a sample that can be measured. *Atomic, molecular spectrometry* and *chromatography*, which together comprise the largest and most widely used groups of techniques, can be further subdivided according to their physico-chemical basis. *Spectrometric techniques* may involve either the *emission or absorption* of *electromagnetic radiation* over a very wide range of energies, and can provide qualitative, quantitative and structural information for analytes from major components of a sample down to ultra-trace levels. The most important atomic and molecular spectrometric techniques and their principal applications are listed in Table.No.2.

Chromatographic techniques provide the means of separating the components of mixtures and simultaneous qualitative and quantitative analysis, as required. The linking of chromatographic and spectrometric techniques, called *hyphenation*, provides a powerful means of separating and identifying unknown compounds.

Electrophoresis's another separation technique with similarities to chromatography that is particularly useful for this parathion of charged species. The principal separation techniques and their applications are listed in Table.No.3.

Analytical methods

An analytical method consists of a detailed, stepwise list of instructions to be followed in the qualitative, quantitative or structural analysis of a sample for one or more analytes and using a specified technique. It will include a summary and lists of chemicals and reagents to be used, laboratory apparatus and glassware, and appropriate instrumentation. The quality and sources of chemicals, including solvents, and the required performance characteristics of instruments will also be specified as will the procedure for obtaining a representative sample of the material to be analyzed. This is of crucial importance in obtaining meaningful results. The preparation or pre-treatment of the sample will be followed by any necessary standardization of reagents and/or calibration of instruments under specified conditions. Qualitative tests for the analyte(s) or quantitative measurements under the same conditions as those used for standards complete the practical part of the method. The remaining steps will be concerned with data processing, computational methods for quantitative analysis and the formatting of the analytical report. The statistical assessment of quantitative data is vital in establishing the reliability and value of the data, and the use of various statistical parameters and tests is widespread. Many *standard analytical methods* have been published as papers in analytical journals and other scientific literature, and in textbook form. Collections by trades associations representing, for example, the cosmetics, food, iron and steel, pharmaceutical, polymer plastics and paint, and water industries are available standards organizations and statutory authorities, instrument manufacturer's applications notes, the Royal Society of Chemistry and the US Environmental Protection Agency are also valuable sources of standard methods. Often, laboratories will develop their own *in-house methods* or adapt existing ones for specific purposes.

Method development forms a significant part of the work of most analytical laboratories, and *method validation and* periodic revalidation is a necessity. Selection of the most appropriate analytical method should take into account the following factors:

- The purpose of the analysis, the required time scale and any cost constraints;
- The level of Analyte(s) expected and the detection limit required;
- The nature of the sample, the amount available and the necessary sample preparation procedure;
- The accuracy required for a quantitative analysis;
- The availability of reference materials, standards, chemicals and solvents, instrumentation and any special facilities;
- Possible interference with the detection or quantitative measurement of the analyte(s) and the possible need for sample clean-up to avoid matrix interference;
- The degree of selectivity available − methods may be selective for a small number of analytes or specific for only one.
- Quality control and safety factors.

MATERIALS AND METHODS

Atorvastatin from Sura labs, Clopidogrel from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK). Acetonitrile for HPLC from Merck,

HPLC method development

Trails

Preparation of standard solution

Accurately weigh and transfer 10 mg of Atorvastatin and Clopidogrel working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.15 ml of Atorvastatin and 1.12ml of Clopidogrel from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was methanol: Water, Methanol: Phosphate buffer and ACN: Water with varying proportions. Finally, the mobile phase was optimized to TEA buffer (pH 4.0), Methanol in proportion 65:35 v/v respectively.

Optimization of Column

The method was performed with various C18columns like Symmetry, X terra and ODS column. Phenomenex Gemini C18 (4.6×250mm) 5µ was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions

Instrument used : Waters Alliance 2695 HPLC with PDA Detector 996 model.
Temperature : 40ºC
Column : Phenomenex Gemini C18 (4.6×250mm) 5µ
Mobile phase : Methanol: TEA Buffer (65:35 v/v)
Flow rate : 1ml/min
Wavelength : 230nm
Injection volume : 10µl
Run time : 6minutes

Validation

Preparation of buffer and mobile phase

Preparation of Triethylamine buffer (pH-4.0)

Take 6.0ml of Triethylamine in to 750ml of HPLC water in a 1000ml volumetric flask and mix well. Make up the volume up to mark with water and adjust the pH to 4.0 by using Orthophosphoric acid, filter and sonicate.

Preparation of mobile phase

Accurately measured 350 ml (35%) of TEA buffer and 650 ml of HPLC Methanol (65%) were mixed and degassed in a digital ultrasonicated for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase ratio : Methanol: TEA Buffer (65:35 v/v)
Column : Phenomenex Gemini C18 (4.6×250mm) 5µ
Column temperature: 40ºC
Wavelength : 230nm
Flow rate : 1ml/min
Injection volume : 10µl
Run time : 6minutes

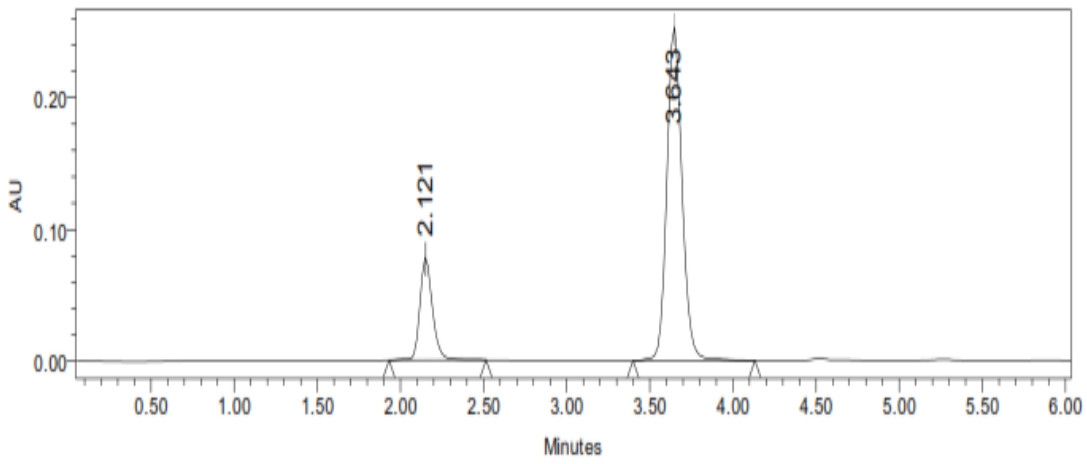

Fig 1: Optimized Chromatogram (Standard)

Table 1: Optimized Chromatogram (Standard)

S.no	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Atorvastatin	2.121	406433	77644	1.2	4009	
2	Clopidogrel	3.643	1592811	251532	1.1	7849	9.8

Optimized Chromatogram

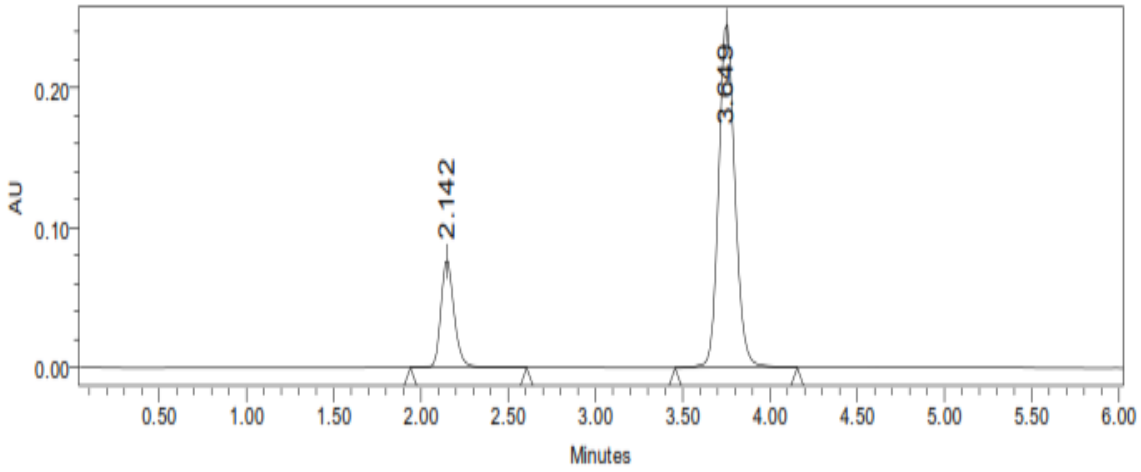

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S.No	Name	Rt	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Atorvastatin	2.142	403871	77464	1.2	4136	
2	Clopidogrel	3.649	1573821	259361	1.1	7812	10.3

- *Resolution between two drugs must be not less than 2*
- *Theoretical plates must be not less than 2000*
- *Tailing factor must be not less than 0.9 and not more than 2.*
- *It was found from above data that all the system suitability parameters for developed method were within the limit.*

System suitability

Table 3: Results of system suitability for Atorvastatin

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Atorvastatin	2.152	382726	70725	5271	1.2

2	Atorvastatin	2.157	382621	70625	5928	1.2
3	Atorvastatin	2.141	389172	70617	5283	1.2
4	Atorvastatin	2.133	384152	70718	5763	1.2
5	Atorvastatin	2.166	389721	70172	6222	1.2
Mean			385678.4			
Std. Dev.			3497.932			
% RSD			0.906956			

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Table 4: Results of system suitability for Clopidogrel

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Resolution
1	Clopidogrel	3.674	1562821	227365	5827	1.1	10.1
2	Clopidogrel	3.631	1562726	226748	6183	1.1	10.1
3	Clopidogrel	3.625	1567361	227163	5029	1.1	10.1
4	Clopidogrel	3.692	1562811	226948	4920	1.1	10.1
5	Clopidogrel	3.629	1563816	226452	5183	1.1	10.1
Mean			1563907				
Std. Dev.			1982.03				
% RSD			0.126736				

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Specificity

Assay (Standard)

Table 5: Peak results for assay standard of Atorvastatin

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Atorvastatin	2.152	406538	77074	1.2	4009	1
2	Atorvastatin	2.198	409975	76001	1.2	4136	2
3	Atorvastatin	2.179	402283	76048	1.2	5263	3

Table 6: Peak results for assay standard of Clopidogrel

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Clopidogrel	3.646	1609924	251956	1.1	7849	1
2	Clopidogrel	3.604	1601840	246020	1.1	7819	2
3	Clopidogrel	3.610	1602832	248287	1.1	7826	3

Assay (Sample)

Table 7: Peak results for Assay sample of Atorvastatin

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Atorvastatin	2.152	406538	77074	1.2	4009	1
2	Atorvastatin	2.150	409975	76001	1.2	4136	2
3	Atorvastatin	2.187	402911	77823	1.2	5173	3

Table 8: Peak results for Assay sample of Clopidogrel

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Clopidogrel	3.646	1609924	251956	1.1	7849	1
2	Clopidogrel	3.651	1601840	246020	1.1	7819	2

3	Clopidogrel	3.601	1603821	240291	1.1	6812	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

=1605195 /1604865 ×10/112.5×112.5/0.0633×99.8/100×0.5382/85×100
= 99.8%
The % purity of Atorvastatin and Clopidogrel in pharmaceutical dosage form was found to be 99.8%

Linearity

Chromatographic data for linearity study of atorvastatin

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	5	135005
66	10	277120
100	15	405128
133	20	534643
166	25	672357

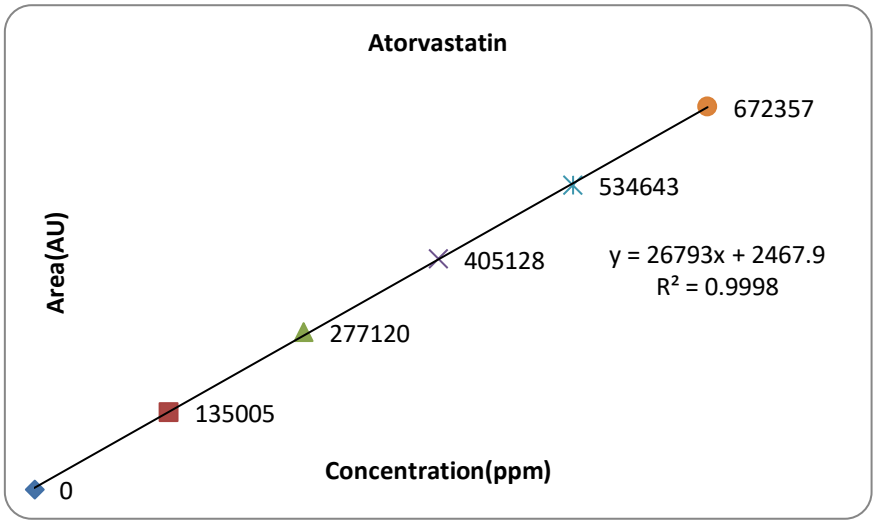

Fig 3: Atovastation

Chromatographic data for linearity study of clopidogrel

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	30	469094
66	75	1149397
100	112.5	1657592
133	150	2150412
166	187.5	2748444

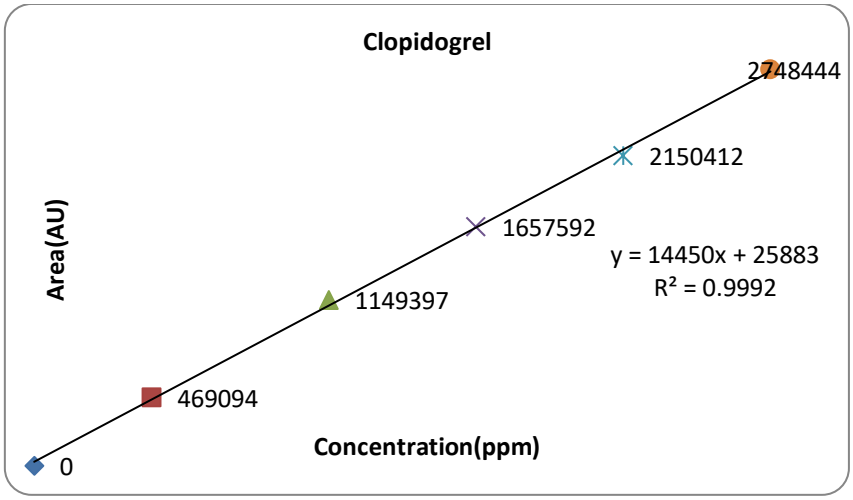

Fig 4: Clopidogrel

Repeatability

Table 9: Results of repeatability for Atorvastatin

S. No	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	%Assay
1	Atorvastatin	2.157	400459	70717	1.2	4987	99%
2	Atorvastatin	2.159	402118	71819	1.2	5019	99.4%
3	Atorvastatin	2.186	405412	73930	1.2	5126	100%
4	Atorvastatin	2.160	406506	73333	1.3	4999	100%
5	Atorvastatin	2.170	407673	72623	1.2	5214	100%
Mean			404433.6				
Std.dev			2716.809				
%RSD			0.671757				

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Table 10: Results of repeatability for Clopidogrel

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing	%Assay
1	Clopidogrel	3.603	1617864	226985	1.1	7045	98.7%
2	Clopidogrel	3.608	1618493	234764	1.1	7399	98.8%
3	Clopidogrel	3.600	1628262	227712	1.2	7159	99.4%
4	Clopidogrel	3.696	1615796	235459	1.1	7896	98.6%
5	Clopidogrel	3.629	1619626	242158	1.1	7965	98.8%
Mean			1620008				
Std.dev			4310.623				
%RSD			0.266086				

Intermediate precision

Table 11: Results of Intermediate precision for Atorvastatin

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	%Assay
1	Atorvastatin	2.198	405262	70572	5672	1.2	100%
2	Atorvastatin	2.196	405637	70516	5639	1.2	100%
3	Atorvastatin	2.160	405628	70572	6183	1.2	100%
4	Atorvastatin	2.160	405647	70372	5923	1.2	100%
5	Atorvastatin	2.160	405948	70592	6739	1.2	100%

6	Atorvastatin	2.186	408732	70526	5837	1.2	100%
Mean			406142.3				
Std. Dev.			1287.197				
% RSD			0.316933				

- *%RSD of five different sample solutions should not more than 2*

Table 12: Results of Intermediate precision for Clopidogrel

S.No	Peak Name	Rt	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution	%Assay
1	Clopidogrel	3.623	1608292	235473	5372	1.1	10.1	98%
2	Clopidogrel	3.611	1609283	235938	5927	1.1	10.1	98.2%
3	Clopidogrel	3.696	1617836	235738	6129	1.1	10.1	98.7%
4	Clopidogrel	3.696	1619743	235963	5284	1.1	10.1	99.7%
5	Clopidogrel	3.696	1614262	231938	5284	1.1	10.1	98.5%
6	Clopidogrel	3.642	1608471	235948	6347	1.1	10.1	98.2%
Mean			1611315					
Std. Dev.			6077.093					
% RSD			0.377151					

- *%RSD of five different sample solutions should not more than 2*

Table 13: Results of Intermediate precision Day 2 for Atorvastatin

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	%Assay
1	Atorvastatin	2.198	405423	70572	5672	1.2	100%
2	Atorvastatin	2.196	405927	70516	5639	1.2	100%
3	Atorvastatin	2.178	405029	70572	6183	1.2	100%
4	Atorvastatin	2.142	405432	70372	5923	1.2	100%
5	Atorvastatin	2.177	405062	70592	6739	1.2	100%
6	Atorvastatin	2.177	408417	70526	5837	1.2	101%
Mean			405881.7				
Std. Dev.			1283.857				
% RSD			0.316313				

- *%RSD of five different sample solutions should not more than 2*

Table 14: Results of Intermediate precision Day 2 for Clopidogrel

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution	%Assay
1	Clopidogrel	3.611	1638732	244384	5363	1.1	10.1	100%
2	Clopidogrel	3.623	1637438	235827	6282	1.1	10.1	100%
3	Clopidogrel	3.684	1638474	236382	5938	1.1	10.1	100%
4	Clopidogrel	3.697	1634273	239183	6194	1.1	10.1	99.7%
5	Clopidogrel	3.684	1636372	231931	5402	1.1	10.1	99.8%
6	Clopidogrel	3.684	1639283	234356	5837	1.1	10.1	100%
Mean			1637429					
Std. Dev.			1860.366					
% RSD			0.113615					

- *%RSD of five different sample solutions should not more than 2*

Accuracy

Table 15: The accuracy results for Atorvastatin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	201472.3	7.5	7.35	98.6	99.7%
100%	406193	15	15.1	100.3	
150%	607144	22.5	22.53	100.2	

- *The percentage recovery was found to be within the limit (98-102%).*

Table 16: The accuracy results for Clopidogrel

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	826527.7	56.25	56.3	101.6	99.6%
100%	1622241	112.5	112.3	99	
150%	2422702	168.75	167	98.9	

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness
Atorvastatin

Table 17: Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	406433	2.121	4009	1.2
Less Flow rate of 0.9 mL/min	398841	2.210	3800.8	0.9
More Flow rate of 1.1 mL/min	389947	2.184	4800.8	
Less organic phase	413898	2.200	4890.8	0.9
More Organic phase	389578	2.172	4190.8	0.7

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Clopidogrel

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1592811	3.643	7849	1.1
Less Flow rate of 0.9 mL/min	1613422	4.498	3312.2	0.9
More Flow rate of 1.1 mL/min	1619138	3.505	4312.2	0.8
Less organic phase	1616104	4.504	4392.2	0.9
More organic phase	1623185	3.512	4292.2	0.9

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Atorvastatin and Clopidogrel in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

Atorvastatin and Clopidogrel are freely soluble in ethanol, methanol and sparingly soluble in water.

Methanol: Triethylamine Buffer was chosen as the mobile phase. The solvent system used in this method was economical.

The %RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

This method can be used for the routine determination of Atorvastatin and Clopidogrel in bulk drug and in Pharmaceutical dosage forms.

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