ejpmr, 2021,8(2), 309-320

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

DEVELOPMENT AND VALIDATION OF A NEW RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ASPIRIN, ATORVASTATIN CALCIUM AND CLOPIDOGREL BISULPHATE IN BULK DRUGS FORMULATION

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Article Received on 23/11/2020 **Article Revised on 12/12/2020** **Article Accepted on 03/01/2021**

ABSTRACT

Introduction: A rapid, accurate, simple andeconomical reverse phase high performance liquid chromatographic method was developed for simultaneous estimation of Aspirin (ASP), Atorvastatin Calcium (ATO) and Clopidogrel Bisulphate (CLO) in bulk drug and pharmaceutical dosage form. **Materials and Methods:** This precise and specific method was operated by adopting the mobile phase containingAcetonitrile: phosphate buffer in ratio 50:50, v/v pH 3.0 which has been adjusted with *o*-phosphoric acid and found to be most suitable for RP-HPLC. The current Separation has been performed on Inertsil ODS-RP-18 column (150 mm x 46 mm) in isocratic mode along with flow rate of 1.2 ml/min and effluents were monitored at 235 nm. **Results:** It shows sharp peak with effective retention time like 1.89 min. (ASP), 6.6 min. (ATO) and 19.8 min. (CLO) respectively. The linearity for ASP, ATO and CLO shows in the range of 30-105 µg/ml, 5-30 µg/ml and 30-105 µg/ml respectively. Recovery results of ASP, ATO and CLO has been found in between of 98.81-100.35 %, 98.28-100.70 % and 99.41-100.61 % respectively. **Conclusion:** This proposed method validation was performed using parameters like accuracy, precision, specificity, linearity, ruggedness and robustness. Current developed PR-HPLC method will be applicable for the simultaneous estimation of these drugs in different pharmaceutical dosage forms due to its specificity and high precision.

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

INTRODUCTION

Chemically, Aspirin is known as 2-(Acetyloxy) benzoic acid. Aspirin is drug which has been orally administered non-steroidal anti-inflammatory agent. It is having ability to suppress the production of prostaglandins and thromboxanes is due to its irreversible inactivation of the cyclooxygenase along with decrease the synthesis of platelet aggregation, and inflammation.[1] it is most commonly used due to their analgesic, antipyretic, and anticoagulant properties. Aspirin produces irreversible inhibition of COX-1 whereas make alteration of the enzymatic activity of COX-2 hence it has been useful in the treatment of a number of conditions, including pain, fever as well as inflammatory conditions like pericarditis, rheumatoid arthritis. It is official in any pharmacopoeia.[2,3]

Atorvastatin calcium is chemically (3*R*,5*R*)-7-[2-(4-Fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]-3,5-dihydroxyheptanoic acid. It exerts lipid-lowering properties and acts as a competitive inhibitor of HMG-CoA reductase and decrease the *de novo* cholesterol synthesis where increasing expression of low-density lipoprotein receptors (LDL receptors) on hepatocytes.[4] The high LDL uptake by hepatocytes minimizes the amount of LDL-cholesterol in the blood. atorvastatin treatment played a plaque-stabilizing role in people who suffering from acute coronary syndrome. Generally atorvastatin is used for the prevention of cardiovascular disease as well as in the treatment of dyslipidemia. It is official in any pharmacopoeia.[5,6]

Clopidogrel sodium which is chemically known as Methyl (+)-(*S*) –α- (*o*-chlorophenyl)-6, 7-dihydrothieno [3,2-*c*] pyridine-5(4*H*)-acetate, sulfate. Generally Clopidogrel is in the form of prodrug and acts as a platelet aggregation inhibitor. It activates in two steps and inhibits $P2Y_{12}$ subtype of ADP receptor specifically as well as irreversibly. [7,8] The platelet aggregation inhibits due to blockade of this receptor by blocking

activation of the glycoprotein IIb/IIIa pathway. Clopidogrel has been used for those people who suffering from heart attack and stroke at high risk. Additionally, it exert properties against myocardial infarction, different forms of acute coronary syndrome along with peripheral artery disease. The use of clopidogrel extended with combination of acetylsalicylic acid (ASA) for the prevention of thrombosis. It is official in any pharmacopoeia.[9,10]

The current research work reports the accurate and precise RP-HPLC method, which validate, develop and calibrates these three active drugs simultaneously in bulk and tablet dosage form. Chromatographic methods have been developed which describes for the individual determination of Aspirin, Atorvastatin calcium and clopidogrel sodium in biological fluids and also as in dosage forms.[11-14] The current method is validated and following the ICH guidelines.[15-17]

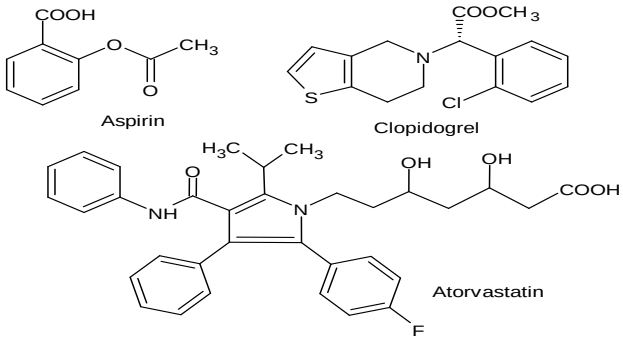

Figure 1: Structures of Aspirin (ASP), Atorvastatin (ATR), Clopidogrel (CLO).

MATERIALS AND METHODS

In this present study, first order derivative spectroscopy and HPLC Methods used for the investigation.

Chemicals and reagents

The pure drug of Aspirin, Atorvastatin calcium, clopidogrel bisulfate used for the current estimation was gifted from Torrent Pharmaceuticals Ltd., (Ahmedabad, India). aspirin and atorvastatin calcium in a commercial capsule Ecosprin AV-75 has been available from Tristar formulation pvt.ltd. All chemicals such as Distilled water (HPLC) were available from a Milli-QRO water purification system. Acetonitrile and Ethanol (HPLC grade), Methanol (AR Grade) and Triethylamine (HPLC Grade) were purchased from Merck Chemicals (Mumbai, India).

Instrumentation

The isocratic elution HPLC system consisted of a pump LC-P-200 (Perkin Elmer Binary Series 200) and UV detector with manual injection facility used to achieve detection where capacity of loop was 20µl. The software used for the current investigation was Total Chrom Navigator version 6.3 where separation was performed on Inertsil ODS -RP-18 column with dimensions 150x46mm. This method analysis of elution was completed at 235 nm on ambient temperature with at flow rate of 1.2 ml/minute. Solution pH has been checked using a digital pH meter (Systronics model EQMK VI). Simultaneous Estimation of compounds through first order derivative UV Spectrophotometric method performed on JASCO UV-V-530 with 1mm quartz cell. For weighing Shimadzu AX200 balance was used. IR spectra of aspirin, atorvastatin calcium, and clopidogrel bisulfate were recorded on Infrared Spectrophotometer model spectrum BX-II (Perkin Elmer, USA).

Chromatographic conditions

Different mobile phases are tried and used on the basis of drug stability, solubility and suitability which have been sonicated for 20 minutes. A suitable MP was selected which consist of Acetonitrile; phosphate buffer (60:40% v/v/v) and optimized for its sensitivity and accuracy for further process. The mobile phase degassed for 15 min and was filtered through 0.45µ membrane hence it avoids the column clogging. Chromatography method has been developed and performed at ambient temperature with constant mobile phase flow rate of 1.5 mL/min. The events were monitored at 290nm. RP-HPLC method was developed and validated for estimation of Aspirin, Atorvastatin calcium and Clopidogrel bisulphate from bulk and capsule formulation. this method was validated by using various parameters such as linearity, precision, accuracy, limit of detection, limit of quantitation, specificity, range and robustness.

Selection of Analytical Wavelength

Stock solutions of drugs were prepared separately in methanol and UV spectrum of 20 µg/ml solutions of aspirin, atorvastatin calcium and clopidogrel bisulphate was taken. For HPLC analysis individual spectra of three drugs were overlain. Wavelength selected for simultaneous analysis was made, that at which all three drugs, exhibited good absorbance.

Preparation of standard stock solution

Standard stock solutions of 100 µg ml^{-1} of aspirin and atorvastatin calcium were prepared by dissolving 10 mg of each in 100 ml of methanol separately. Working standard solutions having concentrations 20 µg ml^{-1} each were prepared by appropriate dilutions.

Preparation of standard calibration curves and selection of analytical Concentration ranges

The standard solutions were prepared by dilution of the stock solution with methanol to reach a concentration range 4-24 µg ml^{-1} for aspirin and atorvastatin calcium. Absorbances were recorded on UV spectrophotometer. The absorbances were plotted against the corresponding concentrations to obtain the calibration graphs.

Analysis of the marketed formulations

Simultaneous UV determination was performed for aspirin and atorvastatin calcium in a commercial capsule Ecosprin AV-75 (Tristar formulation pvt.ltd.) label claim: aspirin (75 mg) and atorvastatin calcium (10 mg), per capsule. EcosprinAVcapsule was dissolved in 100 ml of methanol to get stock solution. The above stock solution was further diluted to get sample solutions concentrations of 30 and 4 µg ml^{-1} of aspirin and atorvastatin calcium respectively and absorbances were recorded on selected wavelengths. The concentrations of two drugs in the mixture were calculated using above equations.

Optimization of HPLC method

The HPLC procedure was optimized with a view to develop a method for simultaneous estimation of aspirin, atorvastatin calcium and clopidogrelbisulphate. Initially different proportions of methanol and water were tried. Trial and error with different proportions of acetonitrile and buffer was carried out until the method was optimized.

Standard solutions and calibration graphs

Stock standard solutions were prepared by dissolving separately 100mg of aspirin, atorvastatin calcium and clopidogrelbisulphate in 100 ml methanol (1000µg/ml). The standard solutions were prepared by dilution of the stock solution with methanol to reach a concentration range 30-105 µg/ml for aspirin, Clopidogrel, bisulphate and 5-30 µg/ml for atorvastatin calcium. Triplicate 20µl injections were made six times for each concentration and chromatographed under the optimized conditions described above. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

Sample preparation for assay

To determine the content of drugs in conventional capsules Ecosporin Gold-10 [(Tristar formulation pvt.Ltd.) and Deplatt- CV (label claim: 75 mg aspirin,10 mg atorvastatin calcium and 75 mg clopidogrelbisulphate per capsule)], the twenty capsules were weighed, their mean weight determined and they were finely powderedand powder equivalent to 75 mg aspirin,10 mg atorvastatin calcium, 75 mg Clopidogrelbisulphate was weighed. Then, equivalent weight of the drug was transferred into a 100 ml volumetric flask containing 50 ml methanol, sonicated for 30 min and diluted to 100ml with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min. Supernatant was taken and after suitable dilution the sample solution was then filtered using 0.45µ filter.

Method Validation of Analytical Methods Developed

Linearity and Range

Linearity of the method was studied by injecting six concentrations of the drug prepared in the mobile phase at the range of 30-105 µg/ml for aspirin, Clopidogrel, bisulphate and 5-30 µg/ml for atorvastatin calcium in triplicate into the HPLC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

Precision

Precision of the method was verified by repeatability and intermediate precision studies. The repeatability of sample application and measurement of peak area for active compounds were expressed in terms of %RSD (relative standard deviation). Repeatability studies were performed by analyses of concentrations 75, 10 µg/ml of aspirin, atorvastatin calcium, clopidogrel bisulphate respectively for HPLC and 1000, 2000, 3000 ng/spot of aspirin, 144, 240, 336 ng/spot of atorvastatin calcium and 720, 1440, 2160 ng/spot of Clopidogrel bisulphate in triplicate for HPTLC on the same day. Intermediate precision of the method was checked by repeating these studies on two different days.

Limit of detection (LOD) and limit of quantitation (LOQ):

The standard deviation of Y-intercept and slope of the calibration curves were used to calculate the LOD and LOQ for all the three drugs using the following formulae.

LOD= 3.3(S)/*s*

LOQ = 10 (S)/ *s*

Table 1: Observation table for Absorption Ratio Method.

Drugs	λ_1 (246.8)	λ_2 (232)
Aspirin (ASP)	Abs. = 0.09072	Abs. = 0.72737 Qx = 8.0158
Atorvastatin calcium(ATV)	Abs. = 0.86991	Abs. = 0.70453 Qy = 0.8098
Mixture	0.3742	1.31123

Robustness of the Method

To evaluate robustness of HPLC method, few parameters were deliberately varied. The parameters included variation of flow rate, percentage of buffer in the mobile phase and pH of mobile phase.

Specificity

Marketed formulations were analysed to determine the specificity of the optimized method in the presence of impurities and excipients.

Accuracy

For HPLC method recovery study was carried out by spiking known amount of standard drug corresponding to 80, 100 and 120% w/w of label claim had been added to marketed drug sample (Standard addition method). At each level of the amount six determinations were performed and the results obtained were compared with expected results.

Analysis of marketed formulation

Assay was performed for aspirin, atorvastatin calcium using commercial capsule (Ecosporin AV): label claim: aspirin (75 mg) Atorvastatin calcium (10 mg), per capsule. The percent purity of aspirin and atorvastatin calcium in the capsule were calculated.

Table 2: Analysis of Aspirin and Atorvastatin calcium in capsule formulation.

Method	Capsule component	Label claim (mg/cap)	Percent Purity	S.D	%R.S.D
Simultaneous Equation method	Aspirin	75	100.3	0.15275	0.15094
	Atorvastatin calcium	10	102	0.64291	0.6319
Absorption ratio method	Aspirin	75	100.5	0.21213	0.2105
	Atorvastatin calcium	10	101	0.4509	0.4442

S.D. = Standard deviation R.S.D. = Relative standard deviation

Assay limit of aspirin in Indian Pharmacopoeia is not less than 99.5 % and not more than 100.5 %. And for atorvastatin calcium not less than 98 % and not more than 102 %. So the obtained results were within the limits prescribed by IP.

Mobile phase Optimization

Different mobile phases were tried in order to find the best conditions for separation of Aspirin, Atorvastatin Calcium, and ClopidogrelBisulphate in ternary mixture. Different mobile phases were tried

Table 3: Mobile phase Optimization.

Column	Mobile phase composition	Concentration (µg/ ml)	Flow rate ml/min.	Retention time	Tailing Factor	Peak Description
HI-Q Sil4.6 mm X250mm	Acetonitrile: Water 50 : 50(V/V)	150:20: 150	1.00	1.79:2.9:--	1.7:1.3:--	Tailing

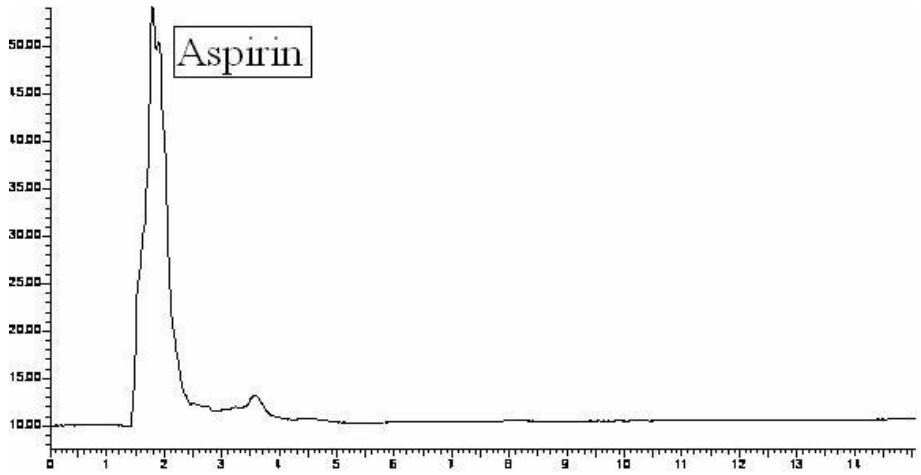

Figure 2: Mobile phase optimization Acetonitrile: Water (50:50).

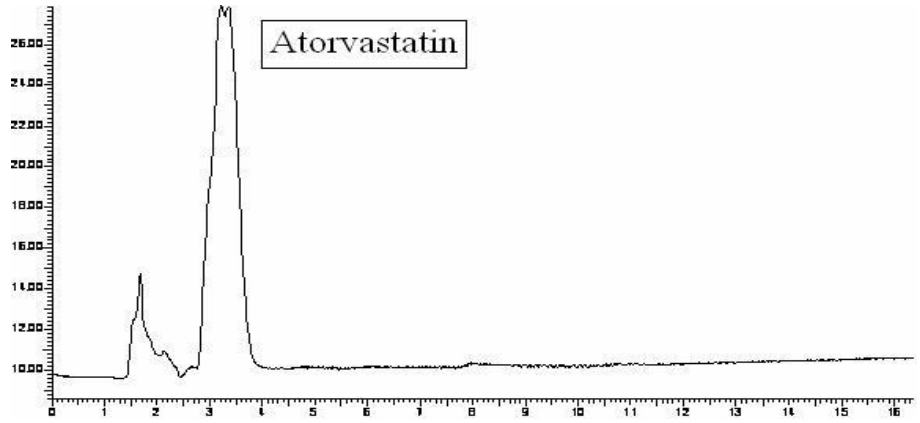

Figure 3: Mobile phase optimization Acetonitrile: Water (50:50).

Mobile phase: Acetonitrile: phosphate buffer in ratio 50:50, v/v pH 3.0adjusted with *o*-phosphoric acid at **λmax of** 235nm with 1.2 ml/min **Flow rate and having** **Retention time** 1.89, 6.6, 19.8 for aspirin, atorvastatin calcium andclopidogrelbisulphate respectively.

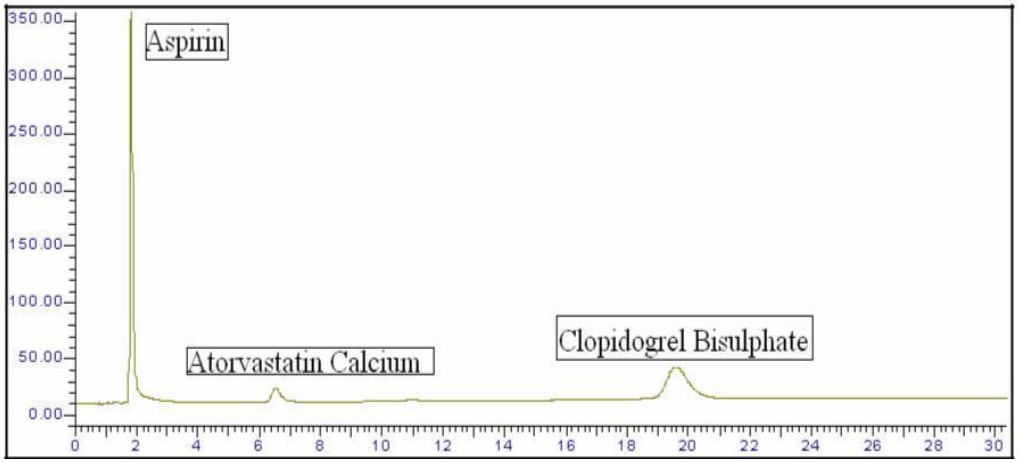

Figure 4: Chromatogram of mixture of ASP, ATO and CLO

Table 4: System suitability parameters: n = 6.

Sr. No.	Parameters	Aspirin	Atorvastatin Calcium	Clopidogrel bisulphate
1.	Retention time	1.89	6.6	19.8
2.	No. of thereotical plates	>2500	>2000	>2000
3.	Tailing factor	0.7	1.2	1.1

Assay of marketed formulation

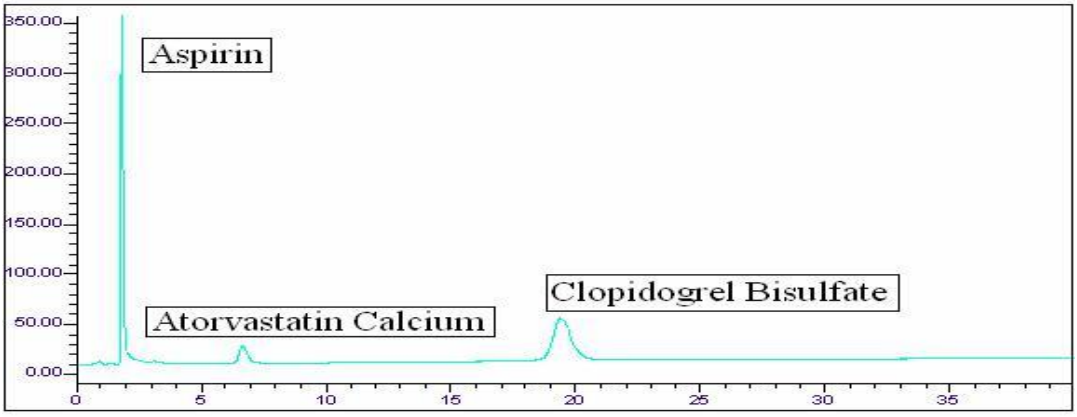

Figure 5: Chromatogram of Assay mixture of ASP, ATO and CLO in Ecosprin Gold-10.

Table 5: Assay of Aspirin, Atorvastatin calcium and Clopidogrel bisulphate *n=6.

Sr. No.	Name of drug	Label claim(mg)	Amount found (mg)	%Drug content*
1	Aspirin	75	74.15	98.87 ±0.24
2	Atorvastatin calcium	10	10.12	101.20 ±0.074
3	Clopidogrelbisulphate	75	73.90	98.53 ±0.43

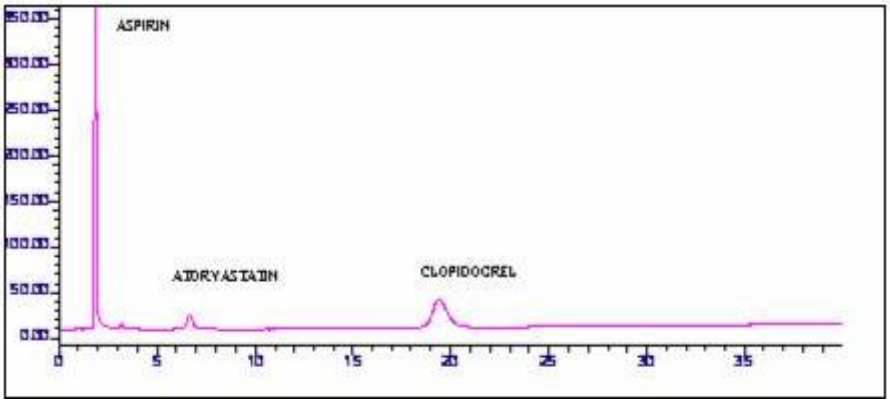

Figure 6: Chromatogram of Assay mixture of ASP, ATO and CLO in Deplatt- CV.

Table 6: Assay of ASP, ATO and CLO *n=6.

S.No.	Name of drug	Label claim (mg)	Amount found (mg)	%Drug content*
1	Aspirin	75	75.15	100.3 ±0.54
2	Atorvastatin calcium	10	9.98	99.80 ±0.22
3	Clopidogrel	75	74.5	99.36 ±0.16

Assay limit of aspirin in Indian Pharmacopoeia is not less than 99.5 % and not more than 100.5 %, for atorvastatin calcium not less than 98 % and not more than 102 % And for clopidogrelbisulphate not less than 97 % and not more than 101.6 %, so the obtained results were within the limits prescribed by IP.

METHOD VALIDATION

Linearity and Range

Linearity was evaluated by determining six standard working solutions of drug twice in triplicate for HPLC.

Table 7: Linearity of Aspirin (n=3).

Standard conc.	30 µg/mL	45 µg/mL	60 µg/mL	75 µg/mL	90 µg/mL	105 µg/mL
Replicates	Peak area					
1	293870	428009	557690	679805	796709	924067
2	298101	428641	553409	674576	790978	927118
3	294124	425674	559347	678747	796643	929576
Mean	295365	427441.3	556815.3	677709.3	794776.7	926920.3
± SD	2372.847	1562.836	3064.105	2764.629	3289.907	2759.814
%RSD	0.8030	0.3656	0.5502	0.4079	0.4139	0.2977

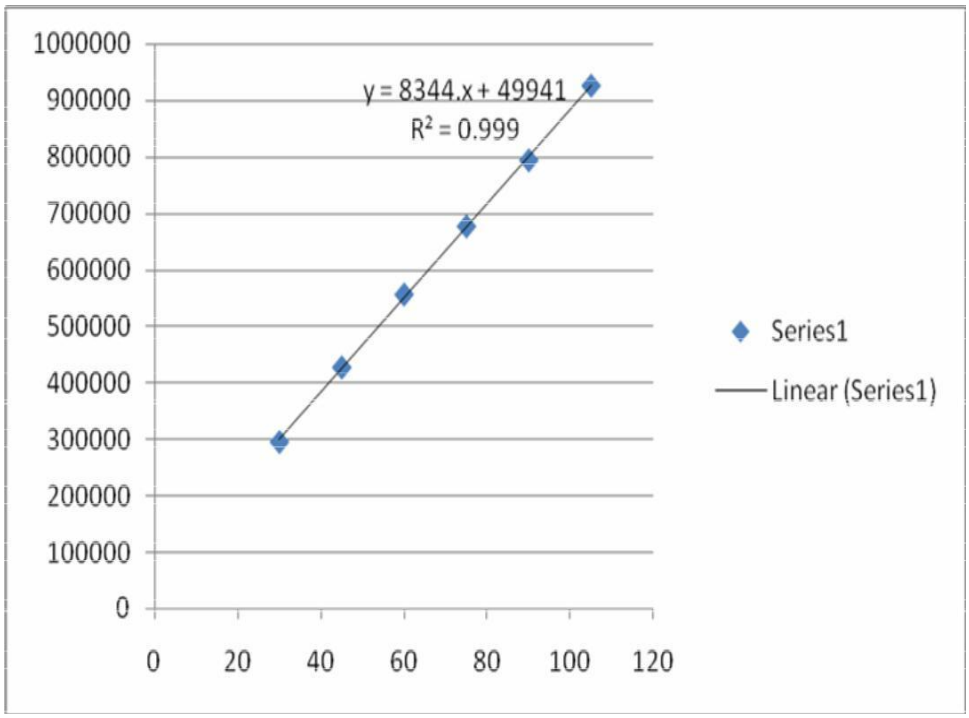

Figure 7: Calibration curve of Aspirin.

Table 8: Linearity of Atorvastatin calcium (n=3).

Standard conc.	5 µg/mL	10 µg/mL	15 µg/mL	20 µg/mL	25 µg/mL	30 µg/mL
Replicates	Peak area					
1	45574	90231	130453	181587	226743	267430
2	43573	90234	137683	182657	227809	265478
3	44895	90769	136509	181432	228790	260913
Mean	44680.67	90411.33	134881.7	181892	227780.7	264607
± SD	1017.573	309.7521	3879.998	667.027	1023.794	3344.668
%RSD	2.2774	0.3426	2.8765	0.3667	0.4494	1.2640

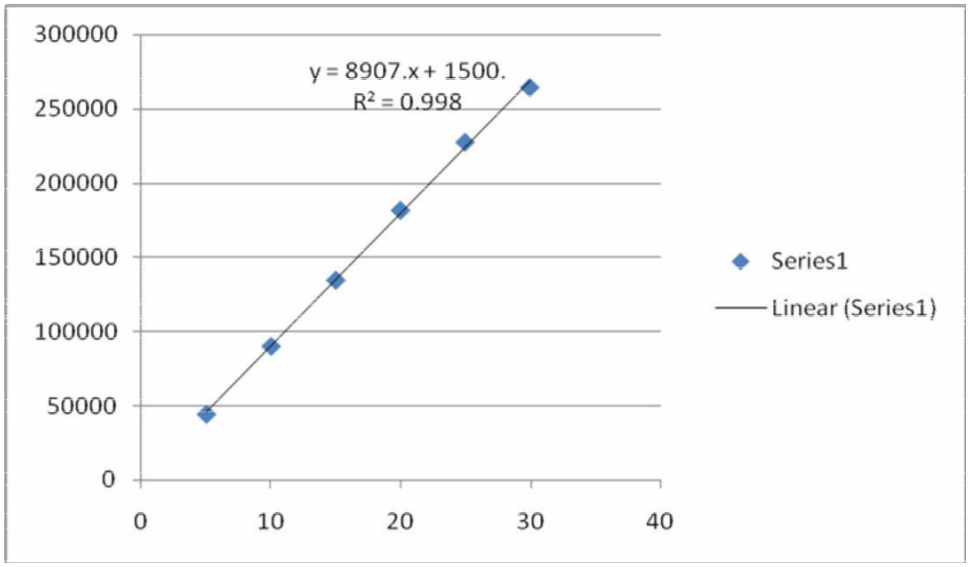

Figure 8: Calibration curve of Atorvastatin calcium

Table 9: Linearity of Clopidogrel bisulfate (n=3).

Standard conc.	30 µg/mL	45 g/mL	60 µg/mL	75 µg/mL	90 µg/mL	105 g/mL
Replicates	**Peak area**					
1	278370	407009	530872	650507	770769	906767
2	271061	406310	535069	657096	774538	906871
3	279424	406046	535487	658747	775443	906653
Mean	276285	406455	533809.3	655450	773583.3	906763.7
± SD	4554.708	497.6053	2552.377	4359.632	2478.933	109.0382
%RSD	1.6485	0.1224	0.4781	0.6651	0.3204	0.0120

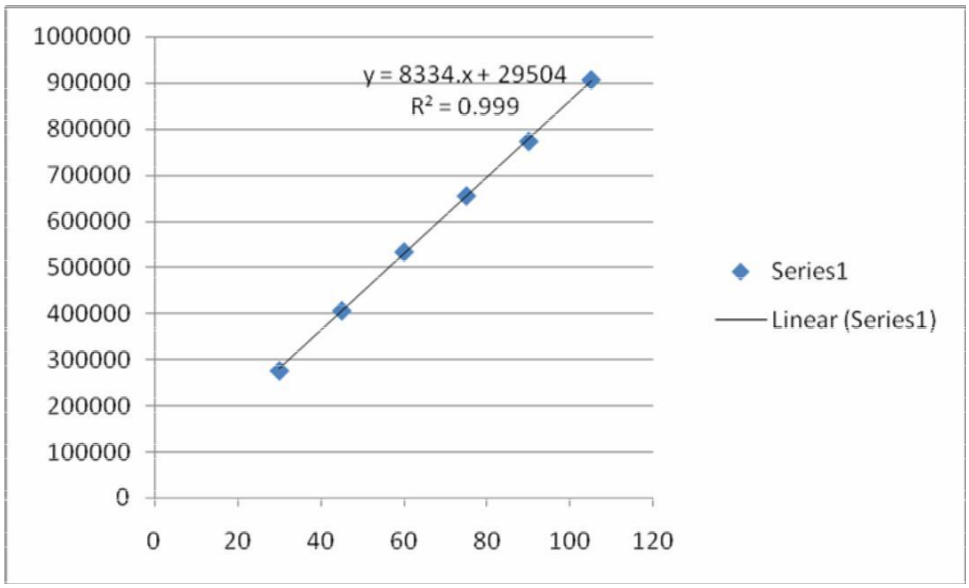

Figure 9: Calibration curve of Clopidogrel bisulfate

Table 10: Linear regression data for calibration curves (n=6).

Parameters	Aspirin	Atorvastatin calcium	Clopidogrel bisulfate
Linearity range µg/ml	30-105	5-30	30-105
r^2 ± SD	0.9994±0.6	0.99836±0.26	0.9991±0.48
Slope	8344	8907	8334
Intercept	49941	1500	29504

r^2 : Correlation coefficient; S.D. : Standard deviation

Calibration curves showed good correlation coefficient in concentration range of 30-105 µg/ml for aspirin, Clopidogrelbisulphate and 5-30 µg/ml for Atorvastatin calcium.

Precision

The within run precision and between-run precision of the developed LC method were determined by analyses of 75µg/ml of the aspirin, Clopidogrelbisulphate and 10µg/ml of atorvastatin calcium on the same day. Intermediate precision of the method was checked by repeating the studies on three different days and expressed in terms of %R.S.D. Low % RSD values for intra and inter day precision confirmed that the method is precise.

Table 11: Intra and Inter day precision for ASP, ATO and CLO.

Compounds	Intra-day precision (n=6)			Inter-day precision (n=6)		
	SD of areas	%RSD	SE	SD of areas	%RSD	SE
Aspirin	0.179	1.51	0.76	0.12	1.03	0.55
Atorvastatin calcium	0.54	1.06	0.39	0.78	1.24	0.68
Clopidogrel bisulphate*	0.82	1.12	0.59	0.50	0.92	0.85

S.D.: Standard deviation, %RSD: Relative standard deviation, SE: Standard error

Repeatability of measurement

Repeatability of measurement of the proposed LC method were determined by analyses of 2000 ng/spot, 240 ng/spot, 1800 ng/spot concentration of the aspirin, atorvastatin calcium, clopidogrelbisulphate respectively.

Table 12: Repeatability of measurement: Aspirin *n=6.

Concentration (ng)	Area		Average Area	% RSD*
	Plate 1	Plate 2		
2000	1472.86	1471.26	1472.06	**0.17**
2000	1472.64	1472.02	1472.33	
2000	1471.46	1472.16	1471.81	
2000	1470.96	1473.06	1472.01	
2000	1471.18	1471.32	1472.053	
2000	1472.22	1471.98	1472.1	

Table 13: Repeatability of measurement: Atorvastatin calcium *n = 6.

Concentration (ng)	Area		Average Area	% RSD*
	Plate 1	Plate 2		
240	953.72	952.30	953.01	**0.87**
240	952.08	951.36	951.72	
240	952.44	953.71	953.075	
240	952.16	952.8	952.48	
240	953.10	951.07	952.085	
240	954.52	953.42	953.97	

Table 14: Repeatability of measurement: Clopidogrel, bisulphate *n = 6.

Concentration (ng)	Area		Average Area	% RSD*
	Plate 1	Plate 2		
1800	1840.62	1841.80	1841.21	0.36
1800	1841.30	1842.06	1841.68	
1800	1842.22	1840.84	1841.53	
1800	1840.78	1841.66	1841.22	
1800	1840.26	1842.08	1841.17	
1800	1841.42	1842.72	1842.07	

%RSD: Relative standard deviation

Table 15: Intra and Inter day precision for Aspirin.

Concentration(ng/µl)	Intra-day precision (n=6)		Inter-day precision (n=6)	
	%RSD	SE	%RSD	SE
1000	0.73	0.32	0.84	0.58
2000	0.17	0.74	0.46	0.55
3000	0.32	0.94	1.03	0.21

Table 16: Intra and Inter day precision for Atorvastatin calcium.

Concentration(ng/µl)	Intra-day precision (n=6)		Inter-day precision (n=6)	
	%RSD	SE	%RSD	SE
144	0.36	0.42	0.56	0.90
240	0.87	0.16	0.62	0.25
336	0.55	0.66	0.44	0.38

Table 17: Intra and Inter day precision for Clopidogrel bisulphate.

Concentration(ng/µl)	Intra-day precision (n=6)		Inter-day precision (n=6)	
	%RSD	SE	%RSD	SE
720	0.69	0.61	0.35	0.83
1440	0.25	0.77	0.64	0.44
2160	0.58	0.11	0.94	0.40

*S.D. : Standard deviation, %RSD : Relative standard deviation, SE : Standard error***.**

LOD and LOQ

Table 18: LOD and LOQ of ASP, ATO and CLO.

Parameter	Aspirin	Atorvastatin Calcium	Clopidogrel bisulphate
LOD (µg/ ml)	1.8	0.0959	0.66
LOQ (µg/ ml)	5.5	0.290	1.89

The LOD and LOQ were found to be 1.8µg/ml, 5.5µg/ml for aspirin, 0.0959 µg/ml , 0.290µg/ml for Atorvastatin Calcium and 0.66 µg/ml, 1.89µg/ml for Clopidogrel, bisulphate.

Robustness

Each factor selected to examine were changed at three levels (-1, 0, 1).One factor at the time was changed to estimate the effect. Insignificant differences in peak areas and less variability in retention time were observed.

Table 19: Robustness of ASP, ATO and CLO.

Factor[b]	Level	Aspirin		Atorvastatin calcium		Clopiogrel bisulphate	
		T_r[c]	T.F	T_r[c]	T.F	T_r[c]	T.F[d]
A: Flow rate							
1.0	-1	1.92	1.1	6.7	0.8	19.86	1.22
1.2	0	1.89	0.8	6.6	0.6	19.8	1.3
1.3	1	1.87	1.4	6.56	1.1	19.72	1.14
Mean ±	-	1.893±	1.1±	6.62±	0.83±	19.79±	1.22
S.D.(n=6)	-	0.025	0.05	0.072	0.035	0.070	±1.10
B: Percentage of buffer in mobile phase(v/v)							
49	-1	1.86	1.2	6.5	0.94	19.7	1.24
50	0	1.89	0.78	6.6	0.9	19.8	1.1
51	1	1.90	1.3	6.66	0.86	19.88	0.96
Mean±	-	1.88±	1.09±	6.58 ±	0.9±	19.81±	1.1±
S.D.(n=6)	-	0.020	0.016	0.080	0.08	0.056	0.21
pH of mobile phase							
2.9	-1	1.86	1.3	6.52	1.06	19.68	0.5
3.0	0	1.89	1.1	6.6	0.58	19.8	1.04
3.1	1	1.94	1.4	6.7	1.3	20.2	0.88
Mean±	-	1.896 ±	1.26±	6.63±	0.98±	19.89	0.78
S.D.(n=6)	-	0.040	0.065	0.133	0.050	±0.27	±0.082

[a] concentration used was 20 µg/ml, [b]Three factors were slightly changed at three different levels (-1, 0, 1), [c] Retention time, [d] Tailing factor

Variation in flow rate, % of buffer in mobile phase, pH did not affect the results. *Rt* and tailing factors of all the three drugs at different levels of variations were similar. Hence, the method was found to be robust.

Specificity

It was observed that single peak for aspirin (Rt, 1.89±0.01), atorvastatin calcium (Rt, 6.6±0.01) and Clopidogrel, bisulphate (Rt 19.8 ±0.01) were obtained under optimized conditions, showing no interference from excipients and impurities. Also the peak areas were compared with the standard and % purity calculated was found to be within the limits.

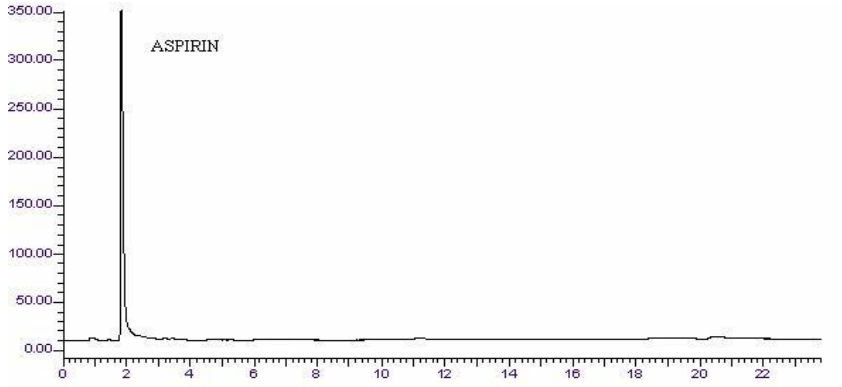

Figure 10: Chromatogram of Aspirin.

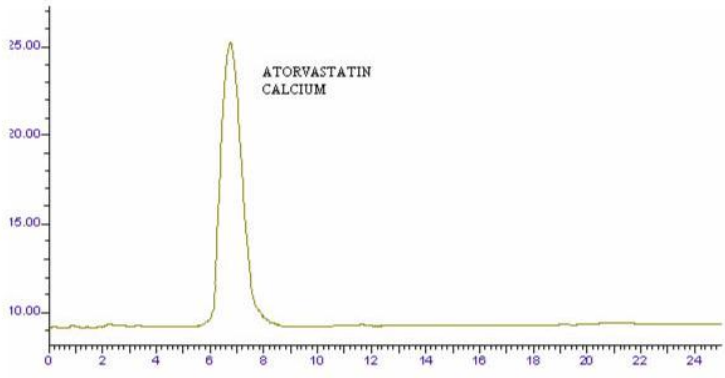

Figure 11: Chromatogram of Atorvastatin calcium.

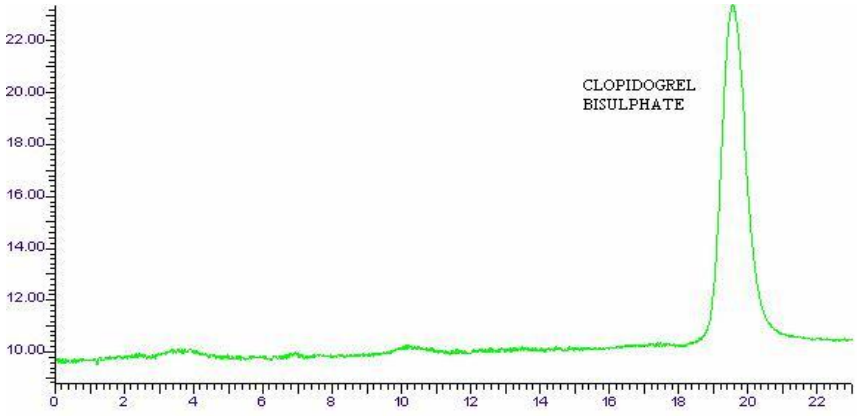

Figure 12: Chromatogram of Clopidogrel bisulphate.

Recovery studies

To ascertain the accuracy of proposed method, recovery studies were carried out by standard addition method, as per ICH guidelines.

Table 20: Recovery studies of ASP, ATO and CLO *n=6.

Label claim (mg/capsule)	Amount added (%)	Total amount added (mg)	Amount recovered (mg)	% Recovery ± SD*	% RSD
Aspirin					
75	80	135	134.47	99.61 ± 0.55	0.56
	100	150	148.22	98.81 ± 0.61	0.28
	120	165	165.12	100.35 ± 0.83	0.53
Atorvastatin calcium					
10	80	18	17.96	98.28 ± 0.49	0.69

	100	20	20.12	100.70 ± 0.26	0.80
	120	22	22.02	100.21 ± 0.27	0.44
Clopidogrel bisulphate					
75	80	135	135.24	100.59 ± 0.25	0.25
	100	150	149.42	99.41 ± 0.11	0.21
	120	165	165.20	100.52 ± 0.33	0.23

S.D.: Standard deviation, %RSD: Relative standard deviation

The % recovery was found to be in the range of 99-100.5% for aspirin, 98-100.7 % for atorvastatin calcium and 99-100.6 % for clopidogrel bisulphate.

RESULT AND DISCUSSION

RP-HPLC Method has been successfully applied and employed for simultaneous estimation of Aspirin, Atorvastatin calcium and Clopidogrel bisulphate from bulk and capsule dosage form. This current developed method was validated as per ICH and USP guidelines by using different parameters like linearity, precision, accuracy, limit of detection, limit of quantitation, specificity, range and robustness.

The linearity of Aspirin, Atorvastatin calcium and Clopidogrel bisulphate has been observed in the range of 30-105 µg/mL, 5-30 µg/mL and 30-105 µg/mL respectively. Detection wavelength used was 235 nm with correlation coefficient 0.9994±0.6 and 0.99836 ±0.26 and 0.9991±0.48 for Aspirin, Atorvastatin calcium and Clopidogrel bisulphate respectively. Retention time of Aspirin was found to be 1.89 min, Atorvastatin calcium shows 6.6 min and Clopidogrel bisulphate was found to be 19.8 min respectively. Quantization was achieved with HPLC detection at 235 nm based on peak area with linear calibration curve.

Thus on the basis of prescribed results these developed methods are precise, accurate, simple, reproducible as well as economical for routine estimation of Aspirin, Atorvastatin calcium and Clopidogrel bisulphate.

CONCLUSION

The current developed method has been successively applicable to pharmaceutical formulation where absence of chromatographic interference from the capsule excipients was found. The RP-HPLC methods were successfully validated as per ICH guidelines in terms of accuracy, precision, recovery as well as robustness. The proposed methods could effectively separate the aspirin, atorvastatin calcium and clopidogrel bisulphate from its mixture.

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