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RP-HPLC METHOD DEVELOPMENT AND VALIDATION STUDIES FOR THE ESTIMATION OF ASPIRIN, CLOPIDOGREL BISULPHATE AND ROSUVASTATIN CALCIUM IN FIXED DOSE COMBINATION CAPSULES

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Keywords:

Aspirin, Rosuvastatin calcium, Clopidogrel bisulphate, RP-HPLC method development, Validation

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ABSTRACT: The present project was conducted with the objective of developing and validating a RP-HPLC method for the simultaneous estimation of aspirin, clopidogrel bisulphate, and rosuvastatin calcium in fixed-dose combination capsule. The chromatographic separation was carried out on Agilent 1260 series using Waters C18 (250 × 4.6 mm, 5 µ) column as the stationary phase and acetonitrile (ACN): phosphate buffer pH 3, gradient mode at a flow rate of 1.2 ml/min and detection at 230 nm. The validation of the developed method was conducted as per the ICH guidelines Q2 (R1). The retention time of aspirin, rosuvastatin calcium and clopidogrel bisulphate was found to be 3.2 min, 4.7 min, and 12.8 min, respectively, under the optimized chromatographic conditions. The developed method was linear in the concentration range of 6.25-400 µg/ml for aspirin, rosuvastatin calcium, and clopidogrel bisulphate. The developed method was specific, with a mean percent recovery of the three drugs in the range of 99-101%. The relative standard deviation (RSD) was less than 2 in the intraday and inter-day precision studies. A simple, accurate, robust, and precise RP-HPLC method was developed for the simultaneous estimation of aspirin, rosuvastatin calcium, and clopidogrel bisulphate in fixed-dose combination capsule. The developed method was validated for linearity, range, accuracy, precision, robustness, LOD, LOQ, and system suitability. This method can be conveniently used for quantification of the three drugs in fixed-dose combination products.

INTRODUCTION: Cardiovascular diseases such as acute coronary syndrome, myocardial infarction, angina, and stroke are considered as a major cause of death and disability in both developed as well as developing countries.

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Various studies have shown that co-administration of aspirin with statins has a synergistic action in the secondary prevention of atherothrombosis and lipophilic statins such as rosuvastatin does not interfere with the antiplatelet effect of clopidogrel in patients with cardiovascular disease.

Fixed-dose combination products containing these drugs are available in the market [1-2]. Aspirin, chemically 2-acetoxybenzoic acid is an odorless, white crystalline powder freely soluble in alcohol, having molecular formula $C_9H_8O_4$, molecular weight 180.2 g/mol and log P of 1.19.

It is a non-steroidal anti-inflammatory agent and an irreversible COX inhibitor with a prominent antiplatelet effect [2-3]. Rosuvastatin calcium, chemically calcium;(E, 3R, 5S)-7- [4(4-fluorophenyl) -2-[methyl (methylsulfonyl)amino]-6-propan-2-ylpyrimidin-5-yl]-3, 5dihydroxyhept-6-enoate is a white crystalline powder soluble in acetonitrile and slightly soluble in acetone, having molecular formula $C_{22}H_{28}FN_3O_6S$, molecular weight 481.53 g/mol and log P of 0.13. It is a statin used to reduce plasma cholesterol levels and prevent cardiovascular disease. It competitively inhibits hydroxymethylglutaryl-coenzyme an (HMG-CoA) reductase, thereby preventing the conversion of HMG-CoA to mevalonic acid, the rate-limiting step in cholesterol biosynthesis [2, 4]. Clopidogrel bisulphate, chemically methyl (2S)-2-(2chlorophenyl)-2-(6,7-dihydro-4H-thieno[3, 2-c]pyridin-5-yl) acetate; sulfuric acid is an off-white powder freely soluble in methanol and practically insoluble in ether, having molecular formula log P of 2.5 **Fig. 1**. It is an oral, thienopyridine-class antiplatelet agent. It prevents platelet activation by irreversibly blocking one of the three adenosine diphosphate receptors (the P2Y12 receptor) on the platelet surface, thus interfering with platelet activation, degranulation, and aggregation [2, 5].

Fixed-dose combination capsules are available in the market bearing the brand name ROSUMAC GOLD. An exhaustive literature survey indicated that many methods were reported for the estimation of rosuvastatin calcium, aspirin, and clopidogrel bisulphate individually and in combination with other drugs [6-16]. However, no HPLC method for the simultaneous estimation of rosuvastatin calcium, aspirin, and clopidogrel bisulphate has been reported so far. Hence, there exists a need to develop and validate a new accurate, precise HPLC method for the simultaneous estimation of rosuvastatin calcium, aspirin, and clopidogrel bisulphate in fixed-dose combination products as per ICH guidelines Q2(R1) [17].

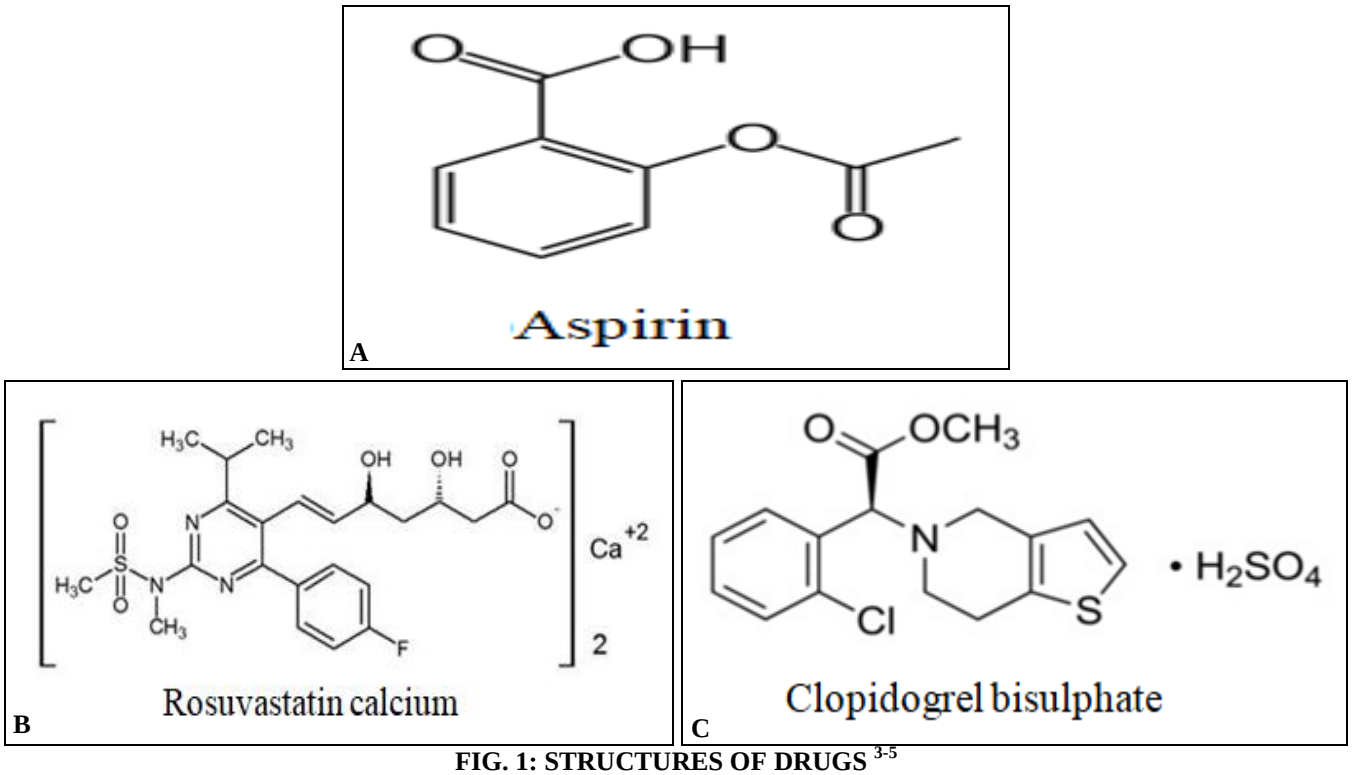

FIG. 1: STRUCTURES OF DRUGS [3-5]

MATERIALS AND METHODS:

Chemicals and Reagents: Reference standards of aspirin, rosuvastatin calcium and clopidogrel bisulphate were received as gift samples from Glenmark Generics Ltd., Mumbai, The Andhra Sugars Ltd. Andhra Pradesh, and Watson Pharma Pvt. Ltd, Ambernath, Maharashtra, respectively. Methanol (HPLC grade) was obtained from S. D. Fine Ltd. Mumbai. Rosumac GOLD capsules containing aspirin (75 mg), rosuvastatin calcium (10 mg), and clopidogrelbislphate (75 mg) were purchased from a local pharmacy store in Mumbai.

Instrument: Analytical method development and validation studies were performed on Agilent Technologies 1260 series chromatograph equipped with a quaternary pump and PDA detector.

Method Development Studies: A variety of mobile phases in the isocratic and gradient mode were evaluated in an effort to arrive at the optimum mobile phase composition capable of resolving the three drugs with good resolution and peak shape within a reasonable run time. The optimum chromatographic conditions comprised of Waters C18 (250 × 4.6 mm, 5 µ) column as the stationary phase and acetonitrile (ACN): phosphate buffer pH 3, gradient mode at a flow rate of 1.2 ml/min and detection at 230 nm.

Preparation of Stock Solutions of Reference Standards: 10 mg of aspirin, rosuvastatin calcium and clopidogrel bisulphate each were accurately weighed and transferred to 10 mL volumetric flask each, and the volume was made up to mark with methanol to give 1000 µg/mL solutions of aspirin, rosuvastatin calcium and clopidogrel bisulphate each.

Preparation of Working Solutions of Reference Standards: Working solutions were prepared by taking 1 mL aliquot of standard stock solution of rosuvastatin calcium (1000 µg/mL), aspirin (1000 µg/mL) and clopidogrel bisulphate (1000 µg/mL) individually and transferring to 10 mL volumetric flask each and making up to mark with methanol to give 100 µg/mL solutions of each drug.

Preparation of Stock and Working Sample Solutions: Ten Rosumac GOLD capsule (Label claim: aspirin 75 mg, rosuvastatin calcium 10 mg, and clopidogrel bisulphate 75 mg) were used. These capsules containing pellets were opened, and the pellets triturated, their average weight recorded. These pellets were finely powdered and weight equivalent to aspirin 75 mg, rosuvastatin calcium 10 mg, and clopidogrel bisulphate 75 mg was taken and transferred to 100 mL volumetric flask. 75 mL of methanol was added and sonicated for 15 min, and volume made up to mark with methanol to give a concentration of 750 ppm aspirin, 100 ppm rosuvastatin calcium, and 750 ppm clopidogrel bisulphate. The sample solution was filtered through Whatman filter paper. 1 mL aliquot of filtrate was taken and transferred to 10 mL volumetric flask and volume made up to mark with methanol to give a concentration of 75 ppm aspirin, 10 ppm rosuvastatin calcium and 75 ppm clopidogrel bisulphate.

Validation Studies: The developed analytical method was validated as per the ICH Q2 (R1) guidelines.

Specificity: Specificity was determined by injecting blank and placebo samples. No peaks were observed at the retention times of aspirin, clopidogrel bisulphate, and rosuvastatin calcium.

Linearity and Range: Calibration curves of the three drugs were prepared at a concentration range of 6.25-400 µg/ml (seven concentration levels) versus the peak area. The linearity was determined using the method of least square regression analysis.

Precision: The precision of an analytical method was studied by performing repeatability, intra-day, and inter-day precision as per the ICH guidelines.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): The limit of detection and limit of quantitation were determined based on the standard deviation of the y-intercept and slope of the regression line from the calibration curves of the three drugs in triplicate. The LOD and LOQ were calculated using the formulae given below:

$$LOD = 3.3\ \sigma / S$$

$$LOQ = 10\ \sigma / S$$

Where, σ is the standard deviation of the response, and S is the slope of the calibration curve.

Robustness: The robustness of the developed method was studied by analyzing the effect of slight variation in the pH of mobile phase (± 0.1 units), change in flow rate (± 0.1 ml/min) and change in mobile phase composition (± 2%) on the retention time, tailing factor, theoretical plates and resolution.

Accuracy: The accuracy of the developed method was determined by calculating the recovery of the three drugs. A fixed concentration of each drug was taken (aspirin-75 µg/ml, rosuvastatin calcium- 10

µg/ml, and clopidogrel bisulphate-75 µg/ml) was taken and the respective reference standard was added at 80%, 100%, and 120% levels. Each level was repeated three times, and the percent recovery and percent relative standard deviation were calculated to estimate the accuracy of the developed method.

System Suitability: The system suitability parameters like retention time, number of USP theoretical plates, USP tailing, peak area, and peak height were evaluated. A standard mixture of aspirin (75 ppm), rosuvastatin calcium (10 ppm), and clopidogrel bisulphate (75 ppm) was injected six times to determine the system suitability of the developed method.

Application of Validated Method for Assay of Fixed-Dose Combination: The assay of the marketed formulation Rosumac GOLD (Label claim: aspirin 75 mg, rosuvastatin calcium 10 mg, and clopidogrel bisulphate 75 mg) was conducted by weighing ten capsules (containing pellets) and determining the average weight. The pellets were finely powdered and weight equivalent to aspirin 75 mg, rosuvastatin calcium 10 mg, and clopidogrel bisulphate 75 mg was transferred to 100 ml volumetric flask. 75 mL of acetonitrile was added and sonicated for 15 min, and volume made up to mark with acetonitrile to give a concentration of 750 ppm aspirin, 100 ppm rosuvastatin calcium and 750 ppm clopidogrel bisulphate.

The sample solution was filtered through Whatman filter paper. 1 mL aliquot of the filtrate was transferred to 10 mL volumetric flask and volume made up to the mark with acetonitrile to give concentration of 75 ppm aspirin, 10 ppm rosuvastatin calcium and 75 ppm clopidogrel bisulphate. 20 µl of this solution was injected, and the chromatogram was recorded and the drug content was calculated from the peak areas.

RESULTS AND DISCUSSION:

Method Development and Optimization: A series of mobile phase compositions were screened in an effort to arrive at an optimum mobile phase that is capable of resolving the three drugs in reasonable run time. The trials were conducted using Waters C18 (250 × 4.6 mm, 5 µ) column as the stationary phase AND various combinations of methanol, ACN, and buffer either alone or combination of two organic solvents with buffer *viz*. ACN: phosphate buffer pH 3: methanol (50: 20:30 v/v/v) which gave low resolution of aspirin (R_t = 2.7 min) and rosuvastatin (R_t = 3.04 min), ACN: phosphate buffer pH 3 (60: 40 v/v) which gave low resolution of aspirin (R_t = 1.6 min) and rosuvastatin (R_t = 2.4 min) and less retention on the column. A range of wavelengths was explored from 220-248 nm before the selection of the optimum wavelength of 230 nm. A range of flow rates from 0.8 to 1.5 ml/min was evaluated before selecting 1.2 ml/min as the optimum flow rate for this method. The optimum chromatographic conditions are given in **Table 1**.

Fig. 2 and **Fig. 3** are the representative chromatograms of a standard mixture of the three drugs and test sample mixture of the three drugs obtained from the capsules.

TABLE 1: OPTIMISED CHROMATOGRAPHIC CONDITIONS OF THE DEVELOPED METHOD

Parameters	Specification
HPLC	Agilent Technologies 1260 series
Stationary phase (column)	Waters C18 (250 × 4.6 mm, 5 µ)
Mode of elution	Gradient
Mobile phase	0-8 minutes- ACN: phosphate buffer pH 3 (50:50) 8-20 minutes- ACN: phosphate buffer pH 3 (75:25)
pH of mobile phase	pH 3 (adjusted using orthophosphoric acid)
Flow rate (ml/min)	1.2 ml/min
Detection wavelength	230 nm
Run time	20 min
Injection Volume	20 µL
Temperature	25 °C

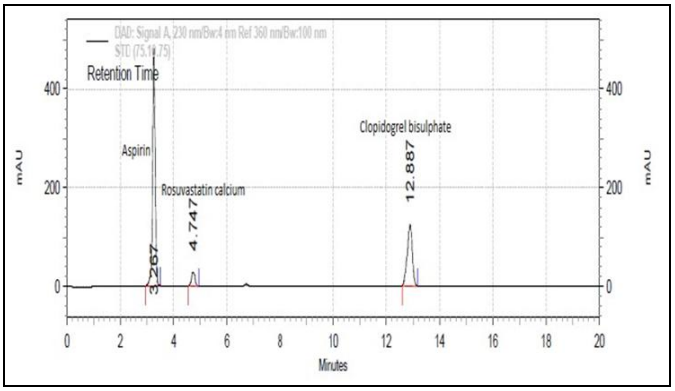

FIG. 2: CHROMATOGRAM OF STANDARD MIXTURE (ASPIRIN 75 PPM, ROSUVASTATIN CALCIUM 10 PPM AND CLOPIDOGREL BISULPHATE 75 PPM)

Validation Studies:

Specificity: No interferences were observed in the chromatogram at the retention times of the three drugs due to the presence of excipients and blank **Fig. 3** indicating that the developed method was specific to the drugs.

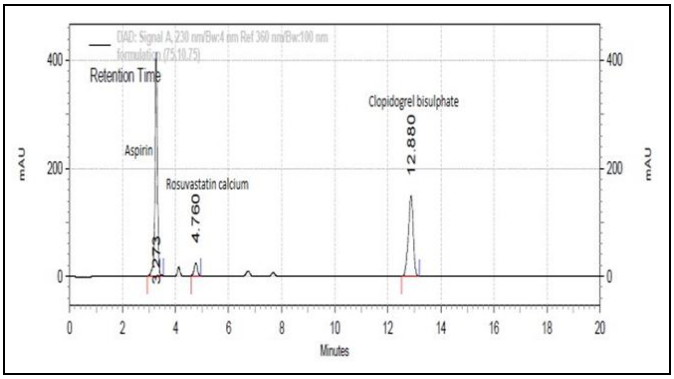

FIG. 3: CHROMATOGRAM OF SAMPLE (ASPIRIN 75 PPM, ROSUVASTATIN CALCIUM 10 PPM AND CLOPIDOGREL BISULPHATE 75 PPM

Linearity and Range: A graph of peak area versus concentration (ppm) was plotted for the three drugs at a concentration range between 6.25-400 ppm, and the response was found to be linear in this concentration range. The linear regression equations and correlation coefficients (r^2) were calculated for the three drugs, and the correlation coefficients were 0.99 to 1.0 for the three drugs **Table 2**. The developed method was found to be linear between 6.25-400 ppm for all the drugs. **Fig. 4**, **Fig. 5** and **Fig. 6** represent the linearity curve of aspirin, rosuvastatin calcium and clopidogrel bisulphate respectively.

LOD and LOQ: The results of the signal to noise ratio was compared with the response of the three drugs. The LOD and LOQ were estimated from the standard deviation of the y-intercepts and slope of the calibration curves of the three drugs.

The LOD of aspirin, rosuvastatin calcium, and clopidogrel bisulphate was found to be 0.96 ppm, 5.36 ppm, and 1.33 ppm, respectively. The LOQ of aspirin, rosuvastatin calcium, and clopidogrel bisulphate was found to be 2.90 ppm, 16.25 ppm, and 4.03 ppm, respectively.

TABLE 2: LINEARITY AND RANGE STUDIES OF THE DEVELOPED METHOD

Concentration* (ppm)	Aspirin mean peak area ± SD	Rosuvastatin calcium Mean peak area ± SD	Clopidogrel bisulphate Mean peak area ± SD
6.25	772376 ± 4737.28	430944 ± 975.14	469755.7 ± 396.21
12.5	1742194 ± 18348.96	764593.7 ± 1405.84	707923.3 ± 3349.39
25	2917426 ± 9068.58	1640190 ± 5690.94	1289152 ± 3422.05
50	6085731 ± 27439.42	3210967 ± 3286.85	2408197 ± 3422.05
100	10736080 ± 177353.6	6402133 ± 5373.97	4670255 ± 758.179
200	20983732 ± 342788.6	12503105 ± 13143.96	9289036 ± 24698.73
400	42315950 ± 724059.4	25212320 ± 140540.3	18650979 ± 26657.48
Regression equation	y = 104606x +360403	y = 62858x + 38691	y = 46216x + 114507
Regression coefficient	0.9997	1	0.9999

*Mean of three determinations

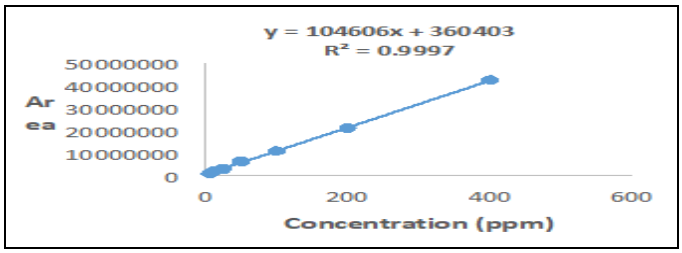

FIG. 4: CALIBRATION CURVE OF ASPIRIN

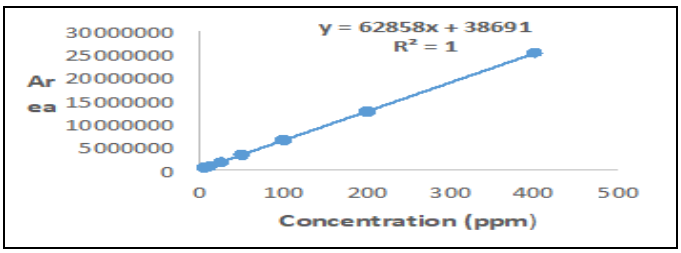

FIG. 5: CALIBRATION CURVE OF ROSUVASTATIN CALCIUM

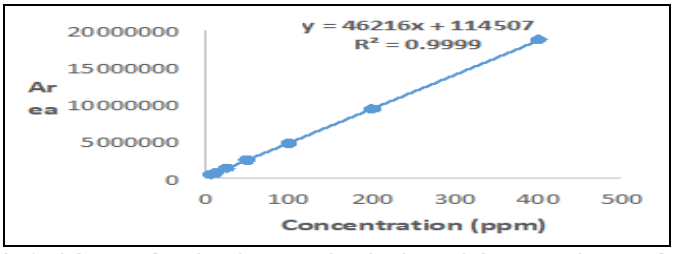

FIG. 6: CALIBRATION CURVE OF CLOPIDOGREL BISULPHATE

Precision: Precision studies comprising of repeatability, intra-day, and inter-day studies were conducted as per the ICH guidelines. Repeatability of the developed method was assessed using aspirin (75 ppm), rosuvastatin calcium (10 ppm), and clopidogrel bisulphate (75 ppm). The intra-day and inter-day precision studies were conducted at three concentrations of the drugs in triplicate. The % RSD was less than 2 for the three drugs indicating that the developed method was precise in **Table 3**.

TABLE 3: PRECISION STUDIES OF THE DEVELOPED METHOD

Concentration (µg/mL) *		Intraday precision *		Inter-day precision *	
		Mean ± SD	%RSD	Mean ± SD	%RSD
Aspirin	75	6741505 ± 24910.15	0.369	6816698 ± 83788.5	1.229
	100	8783033 ± 12094.19	0.137	8770731 ± 17549.23	0.200
	200	17517976 ± 44544.37	0.254	17541929 ± 62182.65	0.354
Rosuvastatin	10	790153.6 ± 8165.72	1.033	789905.9 ± 5932.9	0.751
calcium	25	1647064 ± 26479.64	1.607	1649867 ± 8150.876	0.494
	50	2855796 ± 27197.13	0.952	2749077 ± 39359.04	1.431
Clopidogrel	75	3539136 ± 34063.29	0.962	3687178 ± 40112.72	1.0878
bisulphate	100	4564825 ± 84172.94	1.843	4661908 ± 11677.14	0.2504
	200	9138201 ± 59805.8	0.654	9177773 ± 48572.31	0.5292

Accuracy: The % recovery of aspirin, rosuvastatin calcium, and clopidogrel bisulphate was within 98% 102% of the specified limit, indicating that the developed method was accurate **Table 4**.

TABLE 4: ACCURACY STUDIES OF THE DEVELOPED METHOD

Concentration level *	% Mean Recovery		
	Aspirin	Rosuvastatin calcium	Clopidogrel bisulphate
80 %	100.90	100.16	101.12
100 %	99.36	99.97	99.56
120 %	99.45	100.50	100.12

*Mean of three determinations

Robustness: Small, deliberate changes like pH, flow rate, and mobile phase composition were applied, and their impact on parameters like retention time and tailing factor were determined. The results were found to be within the acceptable limits indicating that the developed method was robust in **Table 5**.

TABLE 5: ROBUSTNESS STUDIES OF THE DEVELOPED METHOD

Robustness Parameters	Drugs	Mean retention time (min)	% RSD	Mean peak area	%RSD	Mean tailing factor	%RSD
pH*							
2.9	Aspirin	3.24	0.83	6626830	0.82	0.89	1.30
	Rosuvastatin calcium	4.69	1.51	481633	1.18	0.96	0.43
	Clopidogrel bisulphate	12.74	0.46	3489272	0.28	0.87	1.57
3.0	Aspirin	3.27	0.19	6686535	0.41	0.96	1.14
	Rosuvastatin calcium	4.75	0.13	496108.3	0.80	1.01	1.14
	Clopidogrel bisulphate	12.90	0.11	3463888	0.84	0.86	1.77
3.1	Aspirin	3.22	0.12	6567677	0.12	0.84	1.28
	Rosuvastatin calcium	4.71	0.22	458798.	0.96	0.99	0.89
	Clopidogrel bisulphate	13.11	0.17	3498869	0.11	0.91	1.81
Flow rate ml/min*							
1.1	Aspirin	3.58	0.02	6760456	0.09	0.984	0.61
	Rosuvastatin calcium	5.14	0.01	835851.3	0.76	0.988893	1.56
	Clopidogrel bisulphate	13.78	0.05	3981666	0.82	0.90	1.53

1.2	Aspirin	3.27	0.19	6183376	0.35	0.96	1.33
	Rosuvastatin calcium	4.74	0.14	772196.7	2.43	0.98	1.45
	Clopidogrel bisulphate	12.9	0.05	3645666	1.05	0.87	0.59
1.3	Aspirin	3.029	0.11	5726830	0.46	0.94	1.56
	Rosuvastatin calcium	4.429	0.07	722660	0.62	0.98	1.81
	Clopidogrel bisulphate	12.2246	0.03	3431867	0.58	0.84	1.05
ACN: phosphate buffer pH 3 (48:52 v/v)	Aspirin	3.417	0.12	6180818	0.16	0.94	1.38
	Rosuvastatin calcium	5.133	0.01	771165.7	0.24	0.97	1.71
	Clopidogrel bisulphate	13.789	0.07	3640089	0.95	0.82	1.26
Mobile phase composition*							
ACN: phosphate buffer pH 3 (50:50 v/v)	Aspirin	3.26	0.12	6154441	0.19	0.96	1.32
	Rosuvastatin calcium	4.76	0.08	760017.7	0.28	0.99	1.25
	Clopidogrel bisulphate	12.88	0.004	3575805	0.77	0.87	0.33
ACN: phosphate buffer pH 3 (52:48 v/v)	Aspirin	3.13	0.1289	6154153	0.03	1.01	1.48
	Rosuvastatin calcium	4.35	0.09	748820	1.27	1.00	1.86
	Clopidogrel bisulphate	12.11	0.05	3634749	0.31	0.91	1.21

System Suitability: The developed method showed theoretical plates above 2000 for the three drugs, tailing factor less than 2 for all the three peaks in the chromatogram and the mean resolution more than 2 between all the peaks **Table 6**.

TABLE 6: SYSTEM SUITABILITY STUDIES OF THE DEVELOPED METHOD

Drugs	Mean retention time (min) *	Mean tailing factor (T) *	Mean theoretical plates (N) *	Mean resolution (R)*
Aspirin	3.27216	0.971188	6133.5	
Rosuvastatin calcium	4.744667	1.000205	7257.333	7.551562
Clopidogrel bisulphate	12.89017	0.875638	19979.83	27.72624
Required limits		T<2	N>2000	R>2

*Mean of six determinations

Application of the Validated Method for the Assay of Fixed-Dose Combination Capsules: The validated method was successfully applied to conduct the assay of marketed fixed-dose combination capsules containing the three drugs. The assay of aspirin, rosuvastatin calcium, and clopidogrel bisulphate was found to be 99.9%, 99.97%, and 99.3%, respectively **Table 7**. The results of the validation studies of the developed method were satisfactory as per the ICH Q2(R1) guidelines confirming that this method can be applied to fixed-dose combination products of these drugs. The developed method is better than the earlier reported methods (UV and HPLC) of fixed-dose combination products containing any two of the three drugs in terms of range, retention time, and accuracy. In addition, the run time of the developed method is short (15 min) with sharp, symmetrical peaks for the three drugs, which increases the number of analyses that can be done.

TABLE 7: ASSAY OF FIXED DOSE COMBINATION CAPSULES

S. no.	Label claim in marketed formulation (mg/capsule)			Amount of drug found (mg/capsule)			% Assay		
	Aspirin	Rosuvastatin calcium	Clopidogrel bisulphate	Aspirin	Rosuvastatin calcium	Clopidogrel bisulphate	Aspirin	Rosuvastatin calcium	Clopidogrel bisulphate
1	75	10	75	74.52	9.92	74.40	99.37	99.29	99.2
2	75	10	75	75.34	9.97	74.65	100.46	99.74	99.54
3	75	10	75	74.90	10.02	74.44	99.87	100.2	99.25
		Mean ± SD		74.92	9.97	74.496	99.9	99.743	99.33
				± 0.41	± 0.05	± 0.134	± 0.545	± 0.455	± 0.183
		%RSD		0.547	0.501	0.18	0.546	0.456	0.184

CONCLUSION: A simple, accurate, precise, and robust RP-HPLC gradient method was developed for the simultaneous estimation of aspirin, rosuvastatin calcium, and clopidogrel bisulphate in

marketed fixed-dose combination capsules. The developed method was validated as per the ICH guidelines Q2 (R1) for parameters, *viz*. specificity, linearity, range, precision, accuracy, LOD, LOQ, robustness and system suitability. This method can be conveniently applied to quantitative analysis of aspirin, rosuvastatin calcium, and clopidogrelbi sulphate in fixed-dose combination products.

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AUTHOR CONTRIBUTION: Ms. Richa has worked on this project and prepared the manuscript draft. Dr. Tabassum Khan has guided this project and edited the manuscript.

CONFLICTS OF INTEREST: None

REFERENCES:

1. Yusuf S, Reddy S, Ounpuu S and Anand S: Part I: General considerations, the epidemiologic transition, risk factors and impact of urbanization. Global Burden of Cardiovascular Diseases Circulation 2001; 104: 2746-53.
2. McTaggart F and Jones P: Effects of statins on high-density lipoproteins: a potential contribution to cardio-vascular benefit. Cardiovasc Drugs Ther 2008; 22: 321-38.
3. USP 28 NF 23, The official compendia of standards, Official monographs Aspirin, Asian edition 2005; 180-4.
4. IP Rosuvastatin calcium 2014; 3: 2683-5.
5. USP 28 NF 23, The official compendia of standards, Official monographs, Clopidogrel bisulphate, Asian edition 2005; 516-7.
6. Shah DA, Bhatt KK, Mehta RS, Shankar MB, Baldania SL and Gandhi TR: Development and validation of a RP-HPLC method for determination of atorvastatin calcium and aspirin in a capsule dosage form. Ind J Pharm Sci 2007, 69(4): 546-9.
7. Mishra P and Dolly A: Simultaneous determination of clopidogrel and aspirin in pharmaceutical dosage forms. Ind J Pharm Sci 2006; 68(3): 365-8.
8. Pandya CB, Channabasavaraj KP and Shridhara HS: Simultaneous estimation of rosuvastatin calcium and ezetimibe in bulk and tablet dosage form by simultaneous equation method. Int J Chem Tech Res 2010; 2(4): 2140-4.
9. Connors KA: A textbook of pharmaceutical analysis. Wiley India edition, edition 3rd 2010; 221-4.
10. Higson SPJ: Analytical chemistry, Oxford university press, Indian edition, 2015; 111-31.
11. Spiridon AM, Neamtu J, Belu I, Stiolica AT and Croitoru O: Simultaneous analysis of clopidogrel bisulphate, acetylsalicylic acid and atorvastatin calcium in tablets by HPLC method. Curr Health Sci J 2015; 41(2): 172-8.
12. Kumar SS, Jamadar LD, Bhat K, Musmade PB, Vasantharaju SG and Udupa N: Analytical method development and validation for aspirin. Int J Chem Tech Res 2010; 2(1): 389-99.
13. Shrivastava PK, Basniwal PK, Jain D and Shrivastava SK: Concurrent estimation of clopidogrelbisulfate and aspirin in tablets by validated RP-HPLC method. Ind J Pharm Sci 2008; 70(5): 667-9.
14. Kaila HO, Ambasana MA, Thakkar RS, Saravaia HT and Shah AK: A new improved RP-HPLC method for assay of rosuvastatin calcium in tablets. Ind J Pharm Sci 2010, 72(5): 592-8.
15. Dumasiya RM, Bhatt KK, Patel B and Joshi NK: Development and validation of stability indicating RP-HPLC method for estimation of rosuvastatin calcium and aspirin in combined dosage form. Int J Pharm Sci 2012; 3(4): 2421-40.
16. Londhe SV, Deshmukh RS, Mulgund SV and Jain KS: Development and validation of a reversed-phase HPLC method for simultaneous determination of aspirin, atorvastatin calcium and clopidogrel bisulphate in capsules. Indian Journal of Pharmaceutical Sciences 2010; 73(1): 23-9.
17. Sudha C, Sohail P and Ushashree A: stability indicating RP-HPLC method for the simultaneous determination of emitricitabine, tenofovir, cobicistat and elvitegravir. International Journal of Pharmaceutical Sciences and Research 2020; 11(3):1452-66.

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