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Stability Indicating Simultaneous Validation of Aspirin, Clopidogrel and Rosuvastatin with Forced Degradation Behavior Study by RP-HPLC in Pharmaceutical Dosage Form

Anurag Mishra[1*], Sanjay Sharma[2], Akhilesh Sharma[1]

[1]Faculty of Pharmaceutical Sciences, Pacific Academy of Higher Education and Research University, Udaipur (Rajasthan), India

[2]School of Pharmacy and Technology Management, SVKM'S, NarseeMonjee Institute of Management Studies, Mumbai-Agra Road, Shirpur-425405, India

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ABSTRACT

For simultaneous validating anassay of Aspirin, Clopidogrel and Rosuvastatin in Capsule Dosage Form, a simple, precise and accurate RP-HPLC method has been developed. Isocratic RP-HPLC process was established on BDS hypersil C_{18} (250mm×4.6mm internal diameter, 5µ particle size) using mobile phase as 0.05M Potassium DihydrogenOrtho Phosphate (pH-4.0): Acetonitrile (60:40v/v) at a flow rate of 1.0 mL/min and at 235 nm the detection was carried out by using tunable absorbance detector (Waters 486). Forced degradation study was performed by acid, base and thermal degradation, as well as oxidation of the drug. The process was validated for accuracy, precision, linearity, and robustness. The method was found to be linear in the 15-45 μg/mL of concentration range with 0.9988, 15-45 μg/mL with 0.9983 and 2-6 μg/mL with 0.9993 of correlation coefficient for Aspirin, Clopidogrel and Rosuastatin, respectively. Degradation products formedbecause of stress studies, did not interfere with the Aspirin, Clopidogrel and Rosuvastatin detection; consequently, the assay can be advised to be stability indicating.

Keywords: HPLC, Aspirin, Clopidogrel and Rosuvastatin, Validation, Forced degradation.

Introduction

Aspirin'sempirical formula is $C_9H_8O_4$and its IUPAC name is 2-acetyloxybenzoic acid[1].Figure 1 shows chemical structure of Aspirin. Chemically Aspirin is a non-selective cyclo-oxygenase inhibitor and used as an antipyretic, analgesic, anti-inflammatory and antithrombotic agent [2-6]. It reduces non-fatal myocardial infarction. Clopidogrel's empirical formula is $C_{16}H_{16}ClNO_2S$ and its IUPAC name is methyl (2S)-2-(2-chlorophenyl)-2-(6,7-dihydro-4H-thieno[3,2-c]pyridin-5-yl)acetate[7,8]. Figure 2 shows the chemical structure of Clopidogrel. It is used an antiplatelet agent, Platelet Aggregation Inhibitor, Fibrinolytic Agents.It is a P2Y12 adenosine diphosphate receptor irreversible inhibitor present on the platelet cells membrane [8-10].

**Correspondence*
Anurag Mishra
School of Pharmaceutical Sciences,
Pacific Academy of Higher Education
and Research University, Udaipur, India
E-Mail: raag.mishra@gmail.com

Rosuvastatin'sempirical formula is $C_{22}H_{28}FN_3O_6S$ as well as its IUPAC name is (E,3R, 5S)-7-[4-(4-fluorophenyl)-2-[methyl(methylsulfonyl)amino]-6-propan-2-ylpyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid.Chemical structure of Rosuvastatin has shown in figure 3.It is an antilipemic agent that competitively inhibits 3- hydroxry- 3-methyl glutaryl coenzyme A (HMGCoA) reductase. HMG-CoA reducutase catalyzes the HMG-CoA to mevalonic acid conversion, a Cholesterol precursor, the rate-limiting step in biosynthesisof cholesterol. The Drug is belonging to a class of medicines which is therapeutically used to decrease a levels of plasma cholesterol and protect from cardiovascular disease as well asreported for the use in the dyslipidemia and hypercholesterolemia treatment[11-14].

These chemical agents are official reported in Indian, British and United States Pharmacopoeia.Previous reportsdisclose that quantitative analysis ofAspirin, Clopidogrel and Rosuvastatin have been done separately on in combination of two and in combination of other drugs but no method is reported for the simultaneous estimation of Aspirin, Clopidogrel

and Rosuvastatin in combined dosage form through HPLC.The present study involved the development and validation of RP-HPLC method for estimateAspirin, Clopidogrel and Rosuvastatin in combined pharmaceutical dosage form and their stability study.

Material and methods

Instruments

The liquid chromatographic system contains Waters series M510 equipped with a tunable absorbance detector (Waters 486), HPLCpump (Waters 510), andmanual injectorrheodynevalve with 20 μL fixed loop.The analysiswasobserved at 235 nm wavelength. Chromatographic analysis was runon Thermo scientificBDS hypersil C_{18}, (250mm × 4.6mm internal diameter, 5µ particle size).Citizen electronic balance were used for weigh measurement of chemicals and drugs.Chemiline India pH meter and ToshconUltrasonicator was used.

Chemicals and reagents

Acetonitrile and Methanol were of HPLC grade obtained from Merk Ltd., Mumbai. Water was of HPLC grade prepared by triple distillation method. Potassium DihydrogenOrtho Phosphate, Ortho Phosphoric Acid (OPA), Sodium Hydroxide (NaOH), Hydrogen Peroxide (H_2O_2) and Hydrochloric Acid (HCl)were of AR grade and were obtained from Merck, Mumbai India.Aspirin (ASP), Clopidogrel (CLP) and Rosuvastatin (ROS) reference standards obtained as gift samples from FDC limited, Mumbai.Capsules as brand name of Rosumac Gold containing 10 mg of Rosuvastatin 75mg ofClopidogrel and 75 mg of Aspirin manufactured by MacleodsPharmaceuticales Ltd. was procured from local market.

HPLC Condition

The mobile phase consisted of 0.05M Potassium Dihydrogenortho Phosphate (pH-4.0): Acetonitrile (60:40v/v).The freshly mobile phase was made and ToshconUltrasonicatorsonicatedwas used for sonication for 5 min before use. BDS hypersil C_{18}, (250mm×4.6mm internal diameter, 5µ particle size) was used and for the period of half an hour it was equilibrated with themobile phase flowing through the system.TheHPLC and column were placed at atmospherictemperature.At 235 nm of wavelength, eluent was observed by UV detection. Analysis was done at flowrate of 1.0ml/min with 20µl volume of injection. All data were analyzed by using Empower 3 software.

Preparation of Mobile Phase

The mobile phase was prepared by mixing 0.05M Potassium DihydrogenOrtho Phosphate (pH-4.0) and Acetonitrile in the ratio of (60:40%v/v). The solution was then filtered through 0.45 microns membrane filter and degassed.

Preparation of 0.05M Potassium Dihydrogen Ortho Phosphate (pH-4.0)

Take about 6.80gm Potasiumdihydrogenortho phosphate into a 1000ml beaker. Add 800ml water and dissolve. Adjust ph 4.0 of this solution with 1% Orthophosphoric acid. Make up volume up to 1000ml with water.

Preparation of standard solution

Standard stock solution of Aspirin, Clopidogrel and Rosuvastatinwere prepared by accurately weighing 30mg, 30mg and 40mg respectively and dissolving them separately in 100ml with methanol to preparesolution of 300μg/mL, 300μg/mL and 400μg/mL. The solution of Rosuvastatin was further diluted by taking 10 ml of standard stock solution and diluted upto 100 ml with methanol separately to prepare solution of40μg/mL.

Forced Degradation Study

Preparation of solution for acid degradation

The study of acid decomposition was made by keeping the working solution of all three drugs (1 ml) in 2 ml of 0.1N HCl for 4.5 hrs. After 4.5hrs solution neutralized with 2ml 0.1N NaOH and finallyprepared up to 10 ml volume with mobile phase, sonicated and filtered byfilter paper of 0.45µm membrane then injected in to HPLC system. Degradation samples were prepared as blank sample, separate standard samples and combined sample of all three drugs.

Preparation of solution for basic degradation

The study of alkali decomposition was done by keeping the working solution of all three drugs (1 ml) in 2 ml of 0.1N NaOH for 5 hrs. 5 hrslater solution neutralized with 2 ml of 0.1N HCL and lastlyprepared up to 10 ml volume with mobile phase, sonicated and filtered by filter paper of 0.45µm membrane then injected in to HPLC system. Degradation samples were prepared as blank sample, separate standard samples and combined sample of all three drugs.

Preparation of solution for oxidative degradation

The study of oxidative decomposition was achieved by keeping the working solution of all three drugs (1 ml) in 2 ml 3% H_2O_2 for 4 hrs. 4hrslater volumeprepared

up to 10 ml with mobile phase, sonicatedthen filteredby filter paper of 0.45µm membrane then injected in to HPLC system. Degradation samples were prepared as blank sample, separate standard samples and combined sample of all three drugs.

Preparation of solution for thermal degradation

The study of thermal decomposition was achieved by refluxing the working solution of all three drugs (1 ml) for 3 hrs at 105 ºC. 4.5hrslater volumeprepared up to 10 ml volume with mobile phase, sonicatedthen filteredby filter paper of 0.45µm membrane then injected in to HPLC system. Degradation samples were prepared as blank sample, separate standard samples and combined sample of all three drugs.

Preparation of solution for UV degradation

UV degradation was done by exposing the working solution of both drugs (1ml) to Sunlight for 210 minutes. 3.5 hours later volume prepared up to 10 ml volume with mobile phase, sonicatedthen filteredby filter paper of 0.45µm membrane then injected in to HPLC system. Degradation samples were prepared as blank sample, separate standard samples and combined sample of all three drugs.

Determination of λ max

The UV spectra of standard stock solutions of Aspirin, Clopidogrel and Rosuvastatintaken between the wave length ranges of 200-400nm using methanol as blank.the λ max was found to be 216.11 nm, 215.39 nm and 243.97 nm for Aspirin, Clopidogrel and Rosuvastatinrespectively. Overlay of the three spectra taken and iso-absorptive point was selected and it was found that all three drugs show appreciable absorbance at 235 nm, so it is used for the further study (Figure 4).

Procedure of Analysis

1ml from Aspirin Standard stock solution, 1ml from ClopidogrelStandard stock solution and 1ml from RosuvastatinStandard stock solution were taken and volume was make up to 10ml with Mobile phase to obtain Working standard solution containing Aspirin (30μg/mL), Clopidogrel (30μg/mL) and Rosuvastatin(4μg/mL).

For Sample stock solution capsule powder was weighed equivalent to 30mg of Aspirin, 30mg of Clopidogrel and 4mg of Rosuvastatin into a 100ml volumetric flask. Add 60ml methanol and shake for 15 minutes. Make up volume with methanol upto 100ml. Filter this solution with whatman filter paper no-1. to prepare sample stock solution of Clopidogrel (300μg/ml), Aspirin (300μg/ml) and Rosuvastatin (40μg/ml). 1ml from Sample stock solution was taken into a 10ml volumetric flask and make up with mobile phase to obtain Working sample solution of concentration Clopidogrel (30μg/mL), Aspirin (30μg/mL) and Rosuvastatin (4μg/mL) respectively.

The contents of standard and sample solution were then filtered through 0.45 µm syringe filter. Chromatograms standard solution (six replicates) was recorded. A typical chromatogram ofAspirin, Clopidogreland Rosuvastatinare presented in figure 5. The retentiontime ofAspirin, Clopidogrel and Rosuvastatinwere 6.89 min, 5.51 min and 10.00 min respectively. The areas of peaks were measured and the quantitation was performed by inserting these values to the regression equation of calibration curve(Table 1).

Optimized Chromatographic Condition:

Stationary phase: Thermo scientific BDS hypersil C_{18} (250mm × 4.6mm, 5µ).

Mobile phase: Potassium dihydrogenortho phosphate (pH 4.0) : Acetonitrile (60:40)

Flow rate :1.0ml/min

Run time (min) :14min

Detection: At 235 nm

Injection (volume) :20µl

Method validation procedure

The developed method was validated for the parameters listed in ICH guidelines.

Linearity

The method was linear in the range of 15-45 μg/mL, 15-45μg/mL and 2-6 μg/mL for Aspirin, Clopidogrel and Rosuvastatin respectively. The linear correlation coefficient for Aspirin, Clopidogrel and Rosuvastatinwere found to be 0.9988, 0.9983 and 0.9993 respectively, and are recorded in table 2, 3 and 4. Calibration curve of Aspirin, Clopidogrel and Rosuvastatin was obtained by plotting the peak area ratio versus the respective concentrations (Figure 6, 7 and 8).

Accuracy

The accuracy of the method was resolved by recovery experiments. Identified amount of working standard was added to the fixed concentration of the pre-analyzed Capsule.By measuring the area before and after the addition of working standard,percent recovery was determined.Recovery was performed in the same wayfor altogetherthree drugs. The recovery studies were carried out in triplicate and outcomes are noted in table 5.This standard addition method was carried out at 80%, 100%, 120% level and the percentage recovery

was determined. Percent recovery was within the range of 98.733% to 99.409% for Aspirin (ASP), 98.803% to 99.165% for Clopidogrel (CLP) and 100.546% to 100.945% for Rosuvastatin (ROS)which shows that the method was accurate.

Precision

For the precision study, repeatability study was carried out for short time interval under the same chromatographic condition. The sample was injected in six replicate. The peak area for all the six replicate was recorded. The mean and % relative standard deviation (%RSD) was calculated and the results are shown in table 6. The %RSD for Aspirin (ASP), Clopidogrel (CLP), Rosuvastatin (ROS) were found to be 0.50%, 0.76% and 1.14% respectively. From the data found the developed RP-HPLC method was found to be precise. For inter-day and intra-day precision three different concentrations (50%, 100% and 150% of analyte) of standard solutions were injected on same day and three consecutive days in three replicates and results were recorded in table 7 & 8.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection and quantification were determinedby standard deviation of response and slope of the calibration curve and results are recorded table 9. The LOD for Aspirin (ASP), Clopidogrel (CLP), Rosuvastatin (ROS) was found to be 2.210µg/ml, 2.614µg/ml and 0.219µg/ml respectively. The LOQ is the lowest concentration of the analyte, which providesresponse that can be exactly quantified. The LOQ for Aspirin (ASP), Clopidogrel (CLP), Rosuvastatin (ROS) was 6.696µg/ml, 7.922µg/ml and 0.664µg/ml.

Robustness

Robustness of the method was analyzed by making minor changes in conditions like mobile phase ratio, pH of buffer, and flowrate in chromatograph and the outcome were documented in table 10. As per observation there were no noticeable changes in chromatograms and % relative standard deviation was found below 2%, which confirmed that the developed RP-HPLC method is robust.

Specificity

The specificity of proposed method is justified by the chromatograms of blank, placebo, standard and sample solutions under same chromatographic conditions shown in figure 9. The placebos did not interfere in determination of Aspirin (ASP), Clopidogrel (CLP), Rosuvastatin (ROS) in commercial Capsule.Specificity of the developed method was also assessed by applying different stress conditions (oxidation, acid, base, thermal and photolytic) to Aspirin (ASP), Clopidogrel (CLP), Rosuvastatin (ROS) Capsule.

Degradation Study

From the results of forced degradation studies showed that these components does not remained intact under stressed conditions and hence special storage conditions should be provided for the dosage form. The specificity studies showed that the principle peaks were well resolved (peak purity 99.99%) and free from any interference from the degradation product. The stress conditions were applied and degraded products of all three drugs are compared and showed in table 10 and chromatograms are in figure 10. From the stress studies it is concluded that substantial degradation of ASP, CLP and ROS occurred in acid, basic, oxidative thermal and photolytic stress conditions. The degradation products (impurities) in addition to percent degradation under acid, base, oxidation, thermal and photolytic stresses have unique retention times (RT) to acidic stress (8 impurities, RT: 2.21 min, 2.58 min, 3.92 min, 5.00 min, 6.15 min, 7.82 min, 8.76 min, and 11.30 min), basic stress (7 impurities, RT: 2.38 min, 2.78 min, 5.03 min, 6.07 min, 7.84 min, 8.76 min and 11.39 min), oxidative stress (8 impurities, RT: 2.32 min, 2.71 min, 3.91 min, 5.05 min, 6.12 min, 7.80 min, 8.74 min and 11.42 min), thermal stress (10 impurities, RT: 2.66 min, 2.88 min, 3.107 min, 4.17 min, 4.99 min, 6.08 min, 6.61 min, 7.91 min, 8.86 and 11.29 min) and photolytic stress (10 impurities, RT: 2.66 min, 2.83 min, 3.12 min, 4.01 min, 4.98 min, 6.07 min, 6.35 min, 7.92 min, 8.88 min and 11.27 min). Degradation studies justified the method specificity for its intended application.

RESULTS AND DISCUSSION

Numerous compositions of mobile phase were tried for developing a novel RP-HPLC method.A suitable separation and good peak symmetry was found with Thermo scientific BDS hypersil C_{18}, 250mm×4.6mm internal diameter, 5µ particle size or equivalent column and mobile phase comprising ofAcetonitrile: Buffer (0.05M potassium dihydrogenortho-phosphate) pH 4.0 with orthophosphoric acid (40:60v/v) at a flow rate of 1.0 ml/min to get improved reproducibility and repeatability. Quantification was performed with UV detection at 235 nm wavelength based on peak area. The retention time forAspirin, Clopidogrel and Rosuvastatin were determined as5.54 min, 6.93 min and 10.04 min, respectively.

According to ICH guidelines the optimized method was validated. The system appropriateness parameters were detected by this optimized condition. The method was found to be linear in the concentration range of 15–45*μ*g/mL with correlation coefficient of 0.9988 for Aspirin, 15–45*μ*g/mL with correlation coefficient of 0.9983 for Clopidogrel and 2–6*μ*g/mL with correlation coefficient of 0.9993 forRosuastatin. The outcomes of recovery study (98.733% to 99.409% for ASP, 98.803% to 99.165% for CLP and 100.546% to 100.945% for ROS) propose that the method has decent recovery. The precision of the projected method was carried in terms of the repeatability. The low% RSD (<2) values of 0.50%, 0.76% and 1.14% variation for Aspirin, Clopidogrel and Rosuastatin respectively, exposes that the projected method is precise. The LOD and LOQ values for Aspirin were found to be 2.210 µg/ml and 6.696 µg/ml, for Clopidogrel were 2.614 µg/ml and 7.922 µg/ml and for Rosuastatin were 0.219 µg/ml and 0.664 µg/ml. The outcomes of robustness in the projected method exhibited no significant changes. The analytical outcomes of dropspecified that no interference due to common excipients was detected with the developed method.Degradation studies justified the method specificity for its planned application.Thus, the proposed method can be used for routine analysis of three drugs in their combined pharmaceutical dosage form.

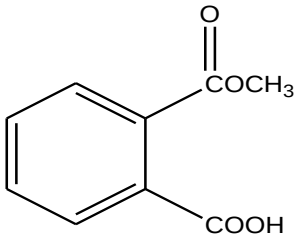


Figure 1: Structure of Aspirin

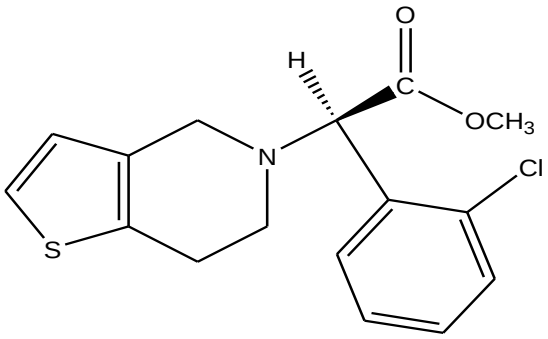


Figure 2: Structure of Clopidogrel

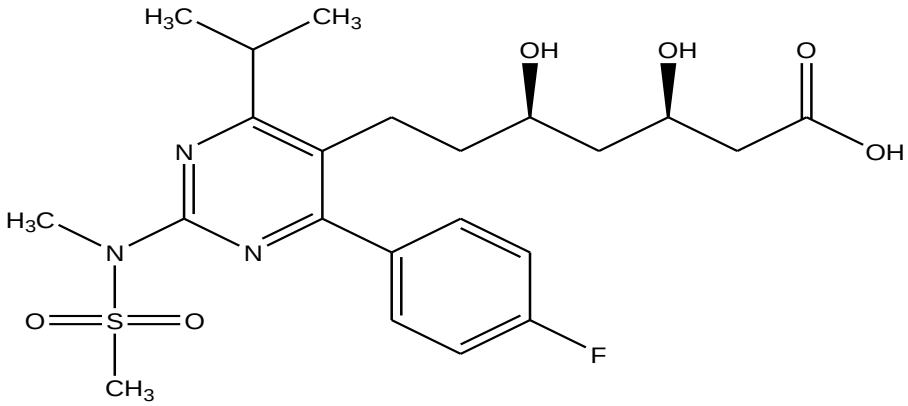


Figure 3: Structure of Rosuvastatin

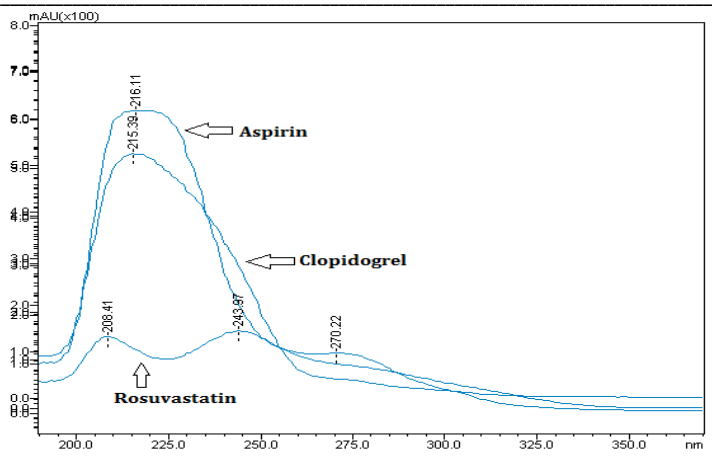


Figure 4: Overlay absorption spectrum for Aspirin, Clopidogrel and Rosuvastatin

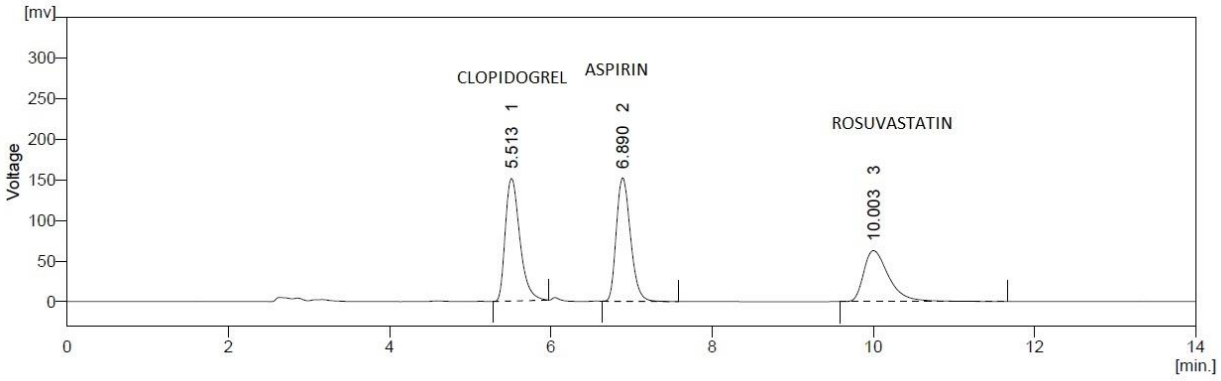


Figure 5: Standard Chromatograms of Aspirin, Clopidogrel and Rosuvastatin

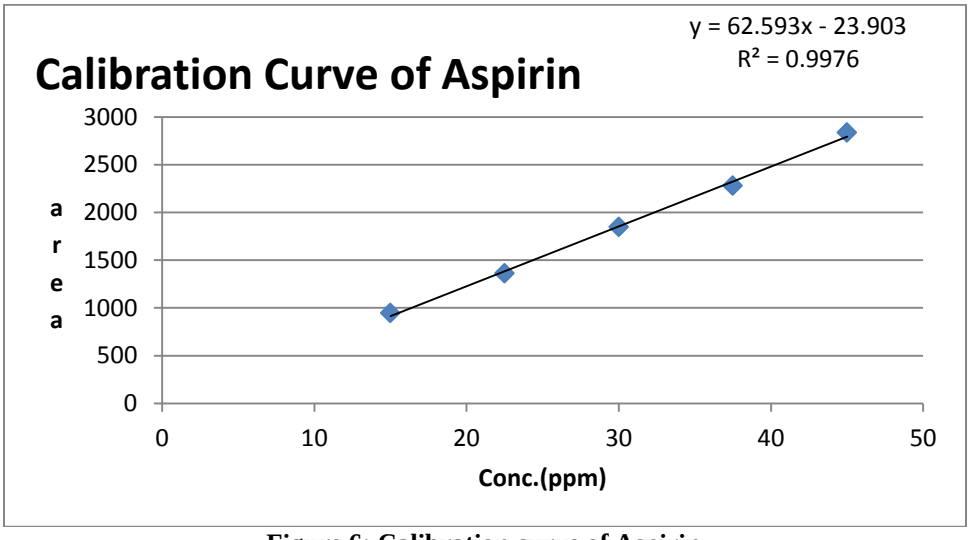


Figure 6: Calibration curve of Aspirin

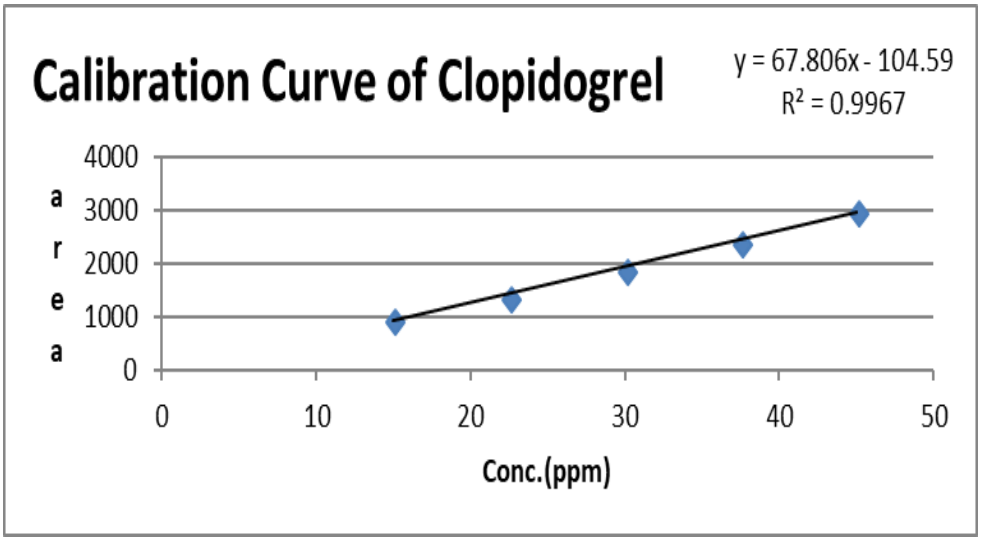


Figure 7: Calibration Curve of Clopidogrel

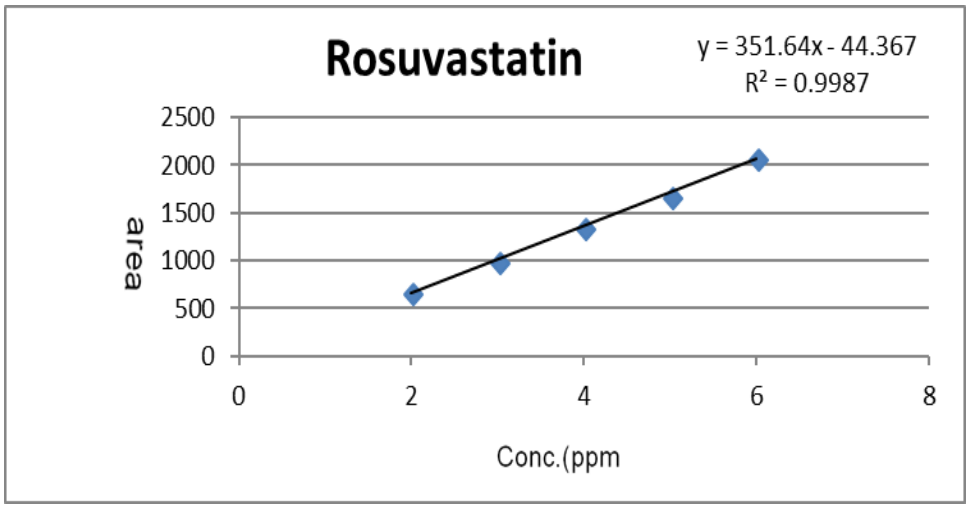
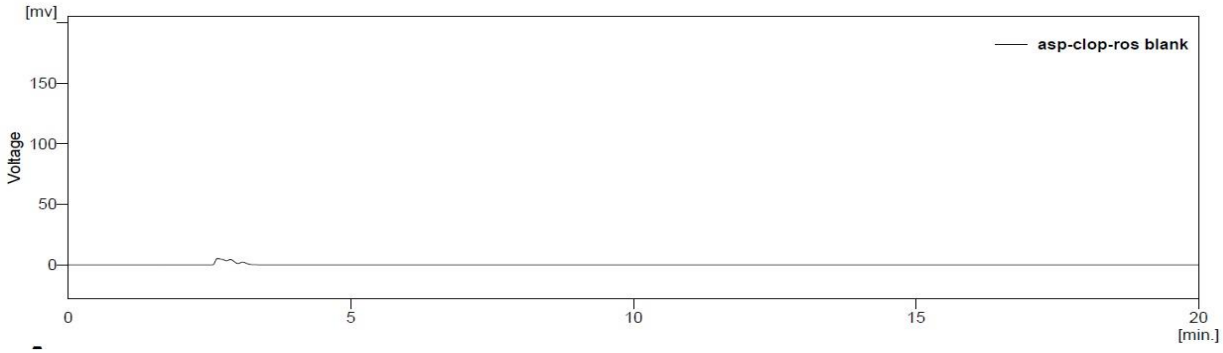
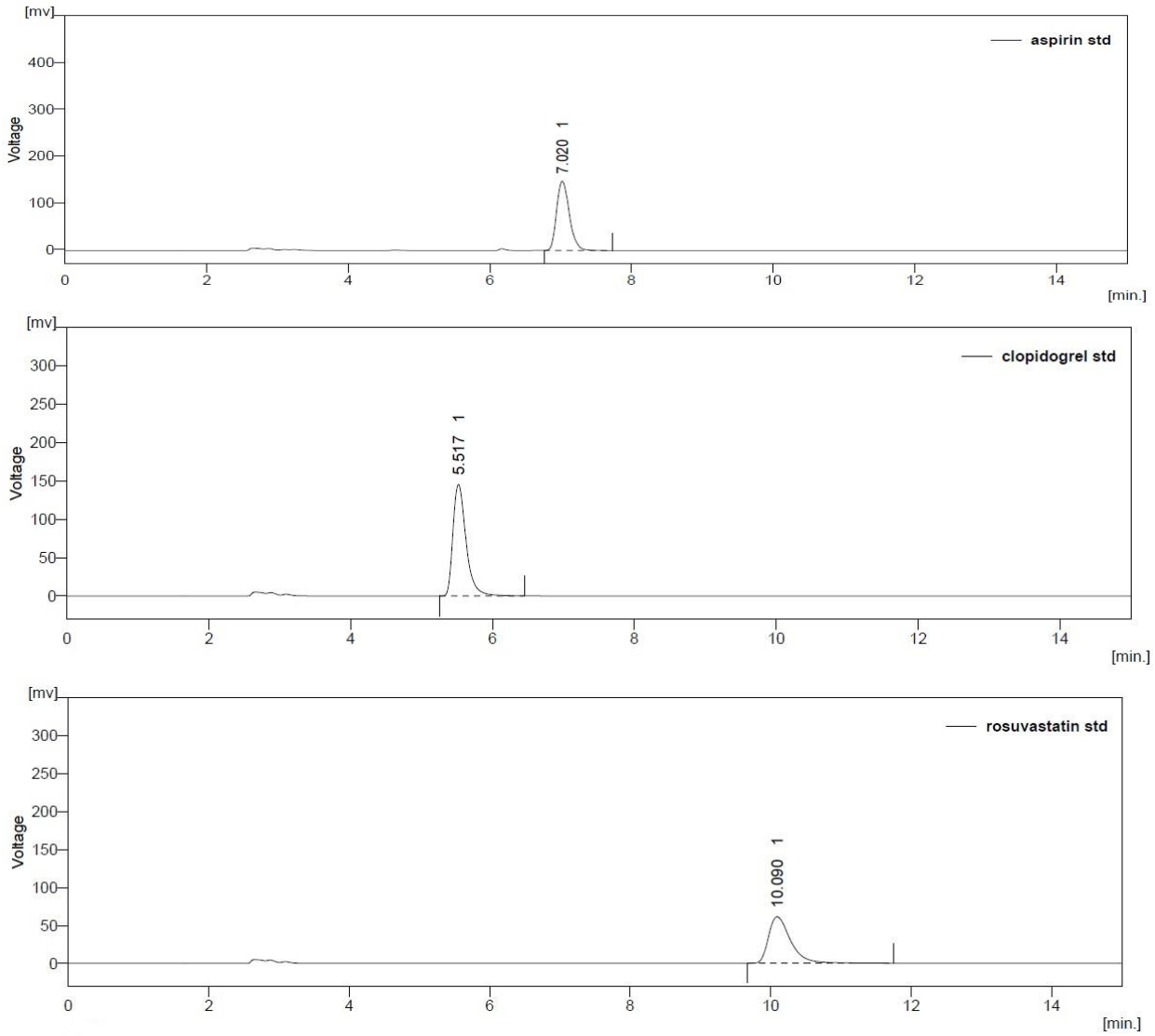


Figure 8: Calibration curve of Rosuvastatin

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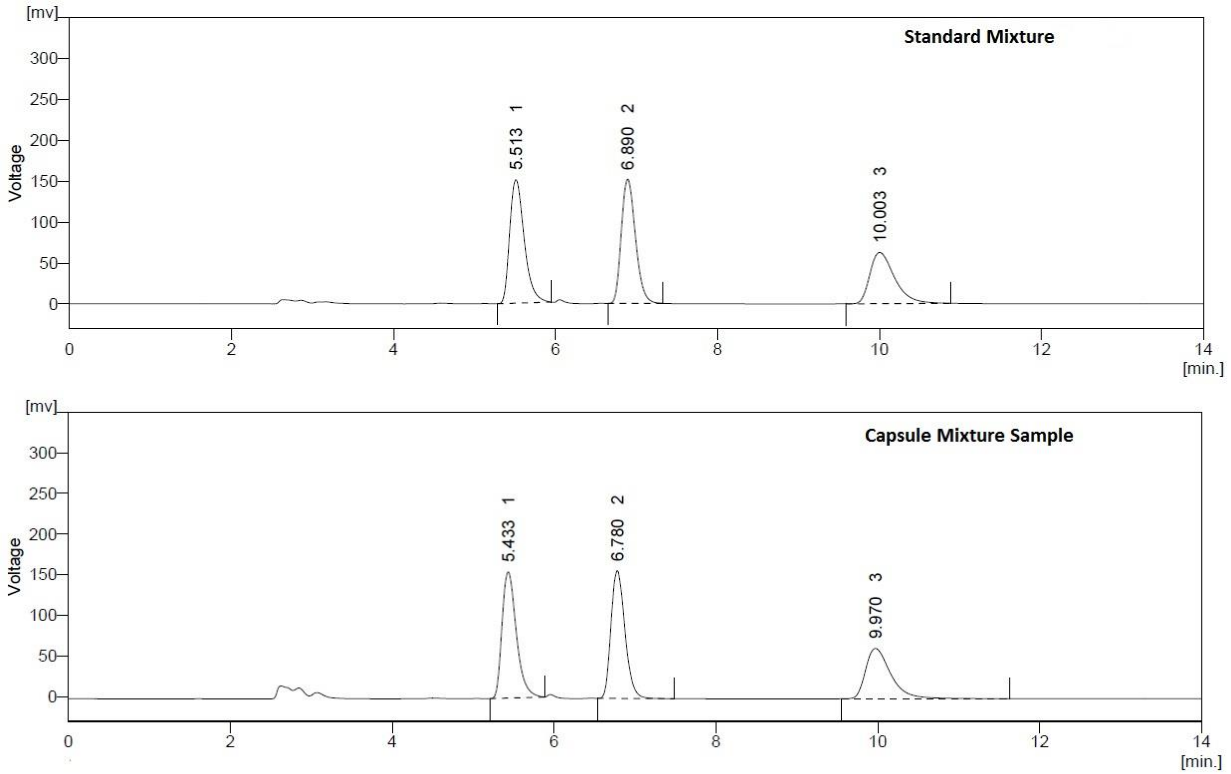
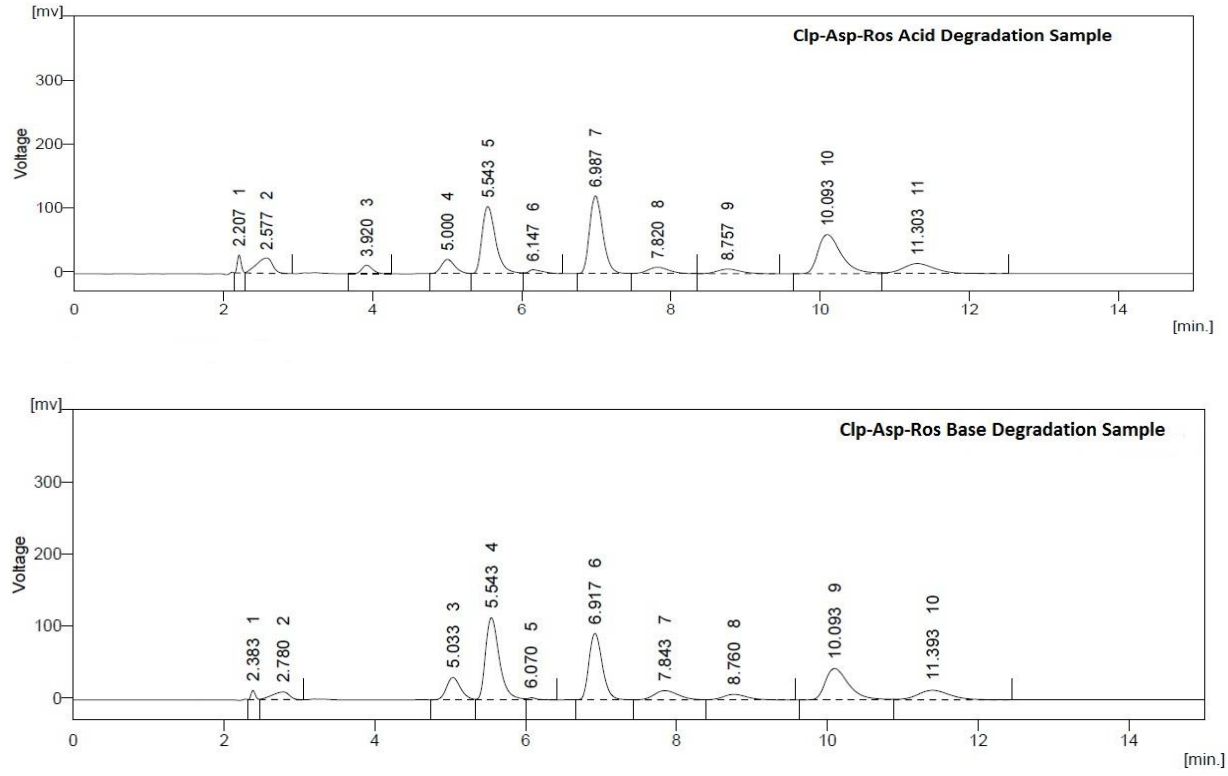


Figure 9: Chromatograms of (a) blank, (b)Aspirin, (c)Clopidogrel,(d) Rosuvastatin, (e) Standard Mixture and (f) Sample Mixture



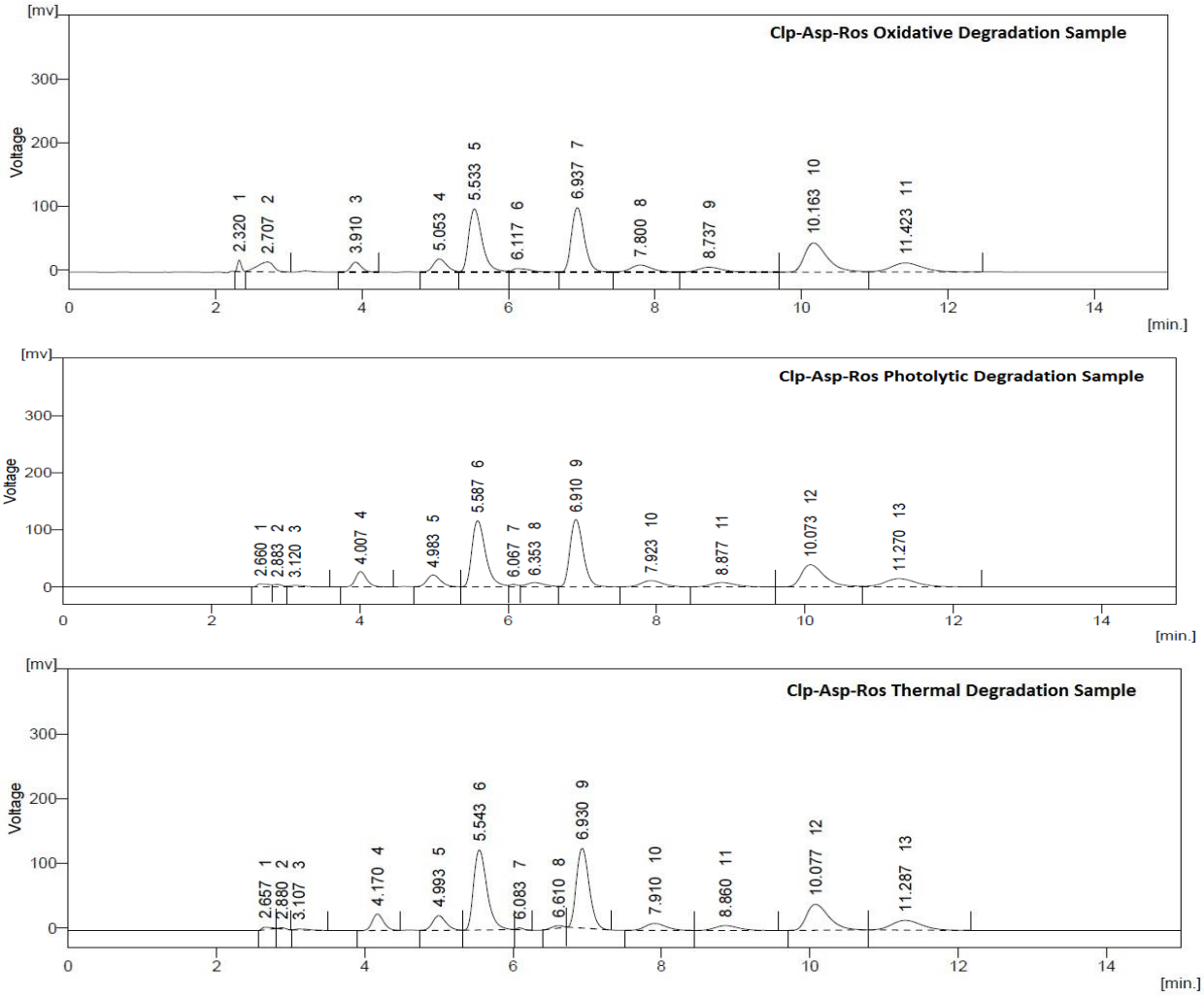


Figure 10: Chromatograms of (a) Acid Degradation, (b) Base Degradation (c) Oxidative Degradation, (d) Thermal Degradation, (e) Photolytic Degradation

Table 1: System suitability of proposed method

Parameters	Aspirin	Clopidogrel	Rosuvastatin
Theoretical plates	7285	4354	5303
Resolution	4.190	--	7.138
Asymmetry	1.442	1.595	1.806
Retention time	6.890 min	5.513 min	10.003 min

Table 2: Linearity results of Aspirin

Linearity Level	Concentration	Area
I	15 µg/ml	945.69
II	22.5 µg/ml	1362.27
III	30 µg/ml	1848.26
IV	37.5 µg/ml	2278.29
V	45 µg/ml	2834.92
Correlation coefficient		0.9988

Table 3: Linearity of Clopidogrel

Linearity Level	Concentration	Area
I	15 µg/ml	966.15
II	22.5 µg/ml	1386.98
III	30 µg/ml	1882.13
IV	37.5 µg/ml	2420.67
V	45 µg/ml	2992.04
Correlation coefficient		0.9983

Table 4: Linearity of Rosuvastatin

Linearity Level	Concentration	Area
I	2 µg/ml	675.55
II	3 µg/ml	999.30
III	4 µg/ml	1358.50
IV	5 µg/ml	1688.31
V	6 µg/ml	2089.22
Correlation coefficient		0.9993

Table 5: Results of Accuracy

Sample	Accuracy	Standard Drug (µg/ml)	Formulation (µg/ml)	% of recovery	S.D.	% RSD
ASP	80%	12	15	99.409	1.226	1.23
	100%	15	15	98.548	0.201	0.20
	120%	18	15	98.733	0.329	0.33
CLP	80%	12	15	99.165	0.588	0.59
	100%	15	15	99.125	0.883	0.89
	120%	18	15	98.803	0.845	0.86
ROS	80%	1.6	2	100.546	1.261	1.25
	100%	2.0	2	100.636	0.579	0.58
	120%	2.4	2	100.945	0.554	0.55

Table 6: Results of Precision

Injection	Area of ASP	Area of CLP	Area of ROS
Injection 1	1896.634	1974.274	1366.159
Injection 2	1913.620	1947.761	1406.088
Injection 3	1906.713	1946.649	1397.305
Injection 4	1897.740	1931.744	1394.359
Injection 5	1905.135	1939.288	1399.834
Injection 6	1921.358	1955.738	1411.659
Average	1906.867	1949.242	1395.901
S.D.	9.4513	14.7172	15.8524
% RSD	0.50	0.76	1.14

Table 7: Result of Inter-day Precision

Conc. (µg/ml)			Area			% RSD		
ASP	**CLP**	**ROS**	**ASP**	**CLP**	**ROS**	**ASP**	**CLP**	**ROS**
15	**15**	**2**	984.591	1001.995	703.244	1.79	0.81	1.63
30	**30**	**4**	1898.106	1936.329	1388.883	0.85	0.55	1.36
45	**45**	**6**	2865.902	2926.618	2105.901	0.76	1.26	0.46

Table 8: Result of Intra-day Precision

Conc. (µg/ml)			Area			% RSD		
ASP	**CLP**	**ROS**	**ASP**	**CLP**	**ROS**	**ASP**	**CLP**	**ROS**
15	**15**	**2**	945.647	969.978	686.509	1.05	1.67	1.77
30	**30**	**4**	1901.478	1956.780	1388.422	0.21	1.78	1.37
45	**45**	**6**	2860.596	2925.940	2094.360	0.41	1.39	0.60

Table 9: Results of LOD and LOQ

Parameter	ASP (µg/ml)	CLP (µg/ml)	ROS (µg/ml)
LOD	2.210	2.614	0.219
LOQ	6.696	7.922	0.664

Table 10: Results of Robustness

Condition	Variation	Average Area			% RSD		
		ASP	**CLP**	**ROS**	**ASP**	**CLP**	**ROS**
Flow rate	0.8 min	2119.549	2162.04	1549.696	0.40	0.61	1.66
	1.2 min	1597.141	1676.466	1167.175	1.57	0.81	0.74
Mobile phase	Buffer: Methanol 62:38	1783.385	1873.256	1294.865	1.48	1.30	0.40
	Buffer: Methanol 58:42	1909.592	2003.498	1398.270	0.72	1.04	1.25
pH	4.2	1947.387	2044.774	1415.070	0.90	1.15	1.06
	3.8	2000.735	2073.360	1464.667	1.46	0.64	1.25

Table 11: Stability study results

Type of degradation	Drug	Peak Area of Standard	Conditions	Peak area			
				Standard		Sample	
				Area of Std.	% Deg.	Area of Sample	% Deg.
Acid degradation	ASP	1841.313	With 0.1N HCL; 4 hours at R.T.	1349.474	26.71	1505.845	18.22
	CLP	1871.457		1302.935	30.38	1337.653	28.52
	ROS	1317.963		1105.702	16.11	1127.165	14.48
Base degradation	ASP	1841.313	With 0.1N NaOH; 4.5 hours at R.T.	1202.563	34.69	1127.425	38.77
	CLP	1871.457		1413.6	24.47	1459.401	22.02
	ROS	1317.963		919.86	30.21	941.735	28.55
Oxidative degradation	ASP	1841.313	With 3% H_2O_2; 4 hours at R.T.	1239.355	32.69	1248.991	32.17
	CLP	1871.457		1206.697	35.52	1268.518	32.22
	ROS	1317.963		1010.498	23.33	996.108	24.42
Thermal degradation	ASP	1841.313	For 4 hours at 105^0C	1517.522	17.58	1475.024	19.89
	CLP	1871.457		1546.737	17.35	1555.102	16.90
	ROS	1317.963		862.096	34.59	838.415	36.39
Photolytic degradation	ASP	1841.313	For 3.5 hours in direct Sun light	1441.711	21.70	1445.592	21.49
	CLP	1871.457		1465.913	21.67	1479.968	20.92
	ROS	1317.963		1057.021	19.80	1031.018	21.77

Conclusion

A simple, precise, accurate and rapid method was developed for simultaneous estimation of Aspirin, Clopidogrel and Rosuastatin from pure and its dosage forms. The preparation of mobile phase is easy and cost-effective. The recoveries of sample in the formulation were in good arrangement with their particular label claims.Henceforth, this method could be effortlessly and suitablyaccepted for routine analysisof Aspirin, Clopidogrel and Rosuastatin in pure form and its dosage form.

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References

1. Fiorucci S, Distrutti E, Mencarelli A, Rizzo G, Lorenzo AR, Baldoni M, et al. Cooperation between aspirin-triggered lipoxin and nitric oxide (NO) mediates antiadhesive properties of 2-(Acetyloxy)benzoic acid 3-(nitrooxymethyl)phenyl ester (NCX-4016) (NO-aspirin) on neutrophil-endothelial cell adherence. The Journal of pharmacology and experimental therapeutics. 2004;309(3):1174-82.
2. Ma L, Cui XL, Wang Y, Li XW, Yang F, Wei D, et al. Aspirin attenuates spontaneous recurrent seizures and inhibits hippocampal neuronal loss, mossy fiber sprouting and aberrant neurogenesis following pilocarpine-induced status epilepticus in rats. Brain research. 2012;1469:103-13.
3. Datta P, Rewers-Felkins K, Kallem RR, Baker T, Hale TW. Transfer of Low Dose Aspirin Into Human Milk. Journal of human lactation : official journal of International Lactation Consultant Association. 2017;33(2):296-9.
4. Zhang JW, Liu TF, Chen XH, Liang WY, Feng XR, Wang L, et al. Validation of aspirin response-related transcripts in patients with coronary artery disease and preliminary investigation on CMTM5 function. Gene. 2017;624:56-65.
5. Costa AC, Reina-Couto M, Albino-Teixeira A, Sousa T. Aspirin and blood pressure: Effects when used alone or in combination with antihypertensive drugs. Revista portuguesa de cardiologia : orgao oficial da Sociedade Portuguesa de Cardiologia = Portuguese journal of cardiology : an official journal of the Portuguese Society of Cardiology. 2017.
6. Al-Jabi SW. Global Trends in Aspirin Resistance-Related Research from 1990 to 2015: A Bibliometric Analysis. Basic & clinical pharmacology & toxicology. 2017.

7. Terrosu P. Aspirin use for primary prevention in elderly patients. Monaldi archives for chest disease = Archivio Monaldi per le malattie del torace. 2016;84(1-2):728.
8. Samant S, Jiang XL, Peletier LA, Shuldiner AR, Horenstein RB, Lewis JP, et al. Identifying clinically relevant sources of variability: The clopidogrel challenge. Clinical pharmacology and therapeutics. 2017;101(2):264-73.
9. Sarafoff N, Byrne RA, Sibbing D. Clinical use of clopidogrel. Current pharmaceutical design. 2012;18(33):5224-39.
10. Trenk D, Nuhrenberg T, Stratz C, Valina CM, Hochholzer W. [Clinical pharmacology of current antiplatelet drugs]. Herz. 2014;39(7):790-7.
11. Desjardins F, Sekkali B, Verreth W, Pelat M, De Keyzer D, Mertens A, et al. Rosuvastatin increases vascular endothelial PPARgamma expression and corrects blood pressure variability in obese dyslipidaemic mice. European heart journal. 2008;29(1):128-37.
12. McKenney JM. Efficacy and safety of rosuvastatin in treatment of dyslipidemia. American journal of health-system pharmacy : AJHP : official journal of the American Society of Health-System Pharmacists. 2005;62(10):1033-47.
13. Xing H, Sun S, Mei Y, Herman D. The protective effect of rosuvastatin on ischemic brain injury and its mechanism. Journal of Huazhong University of Science and Technology Medical sciences = Hua zhong ke ji da xue xue bao Yi xue Ying De wen ban Huazhong keji daxue xuebao Yixue Yingdewen ban. 2006;26(6):667-9.
14. Zipes DP, Zvaifler NJ, Glassock RJ, Gilman S, Munoz A, Gogolak V, et al. Rosuvastatin: an independent analysis of risks and benefits. MedGenMed : Medscape general medicine. 2006;8(2):73.

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