HPLC Method for Determination of Aspirin, Rosuvastatin, Ezetimibe and Clopidogrel in Combination Drug Products

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A single HPLC method for the determination of four active ingredients viz. aspirin, rosuvastatin, ezetimibe and clopidogrel in less run time is developed. HPLC method was developed and validated. X-terra C18 100 × 4.6 mm, 3.5 μ HPLC column, KH₂PO₄ buffer (mobile phase A) and acetonitrile (mobile phase B) were used. 20 μ injection volume, 1.0 mL/min flow rate, 230 nm and ambient column oven temperature were applied for separation. Gradient program: at 0 min mobile phase-B 13 %, at 4 min 13 %, at 8 min 44 %, 14 min 57 %, 17 min 57 %, 20 min 13 % and 25 min 13 %. Method validation was performed as per ICH guidance with precision, linearity, specificity, accuracy, ruggedness and robustness. Validation results were satisfactory and this method can be applied for regular drug product manufacturing.

Keywords: Aspirin, Clopidogrel, Ezetimibe, Rosuvastatin, HPLC method.

INTRODUCTION

Aspirin is used treat pain, inflammation and fever. Aspirin can be given shortly after the heart attack decreases the risk of health and also used for long term to prevent heart attacks, ischaemic strokes and blood clots [1,2]. If we use over dose it may give side effects stomach upset like stomach ulcers, stomach bleeding and asthma [3].

Rosuvastatin belongs to statins group and used for high blood cholesterol, cardiovascular disease. Body LDL cholesterol were reduced and this control effects dose related [4]. Higher dose of rosuvastatin has more efficacious in improving lipid profile of patients with hypercholesterolemia. Few studies were confirmed the moderately increasing of HDL by using rosuvastatin [5]. Side effects are constipation, dizziness, sleeplessness, depression, joint pain, cough, memory loss and heartburn.

Ezetimibe can reduce the cholesterol levels. Ezetimibe is used with statins to achieve the target LDL cholesterol levels [6]. Ezetimibe inhibits the absorption of cholesterol from the small intestine and decreases the quantity of cholesterol. Common adverse effects are headache, diarrhea and myalgia [7]. Clopidogrel reduces the heart disease and heart strokes. Clopidogrel brand name is plavix and also used with aspirin combin-

ation product [8,9]. Clopidogrel is used for acute coronary syndrome, stroke and peripheral artery disease [10].

Chemical structures of aspirin, rosuvastatin, ezetimibe and clopidogrel were represented in Fig. 1. Clopidogrel is available with aspirin and rosuvastatin combinations and rosuvastatin is available with ezetimibe combination dosage form. Table-1 is listed the all available combination products in the market.

Literature survey found less methods to determine the clopidogrel, aspirin and some are reported for ezetimibe and rosuvastatin [11,12] only. Some authors [13-15] reported rosuvastatin and ezetimibe in pharmacokinetic studies. Main objective of this study was to develop a single HPLC method to determine aspirin, rosuvastatin, ezetimibe and clopidogrel in tablets dosage form.

EXPERIMENTAL

A high performance liquid chromatograph of Agilent make 1100 series auto sampler instrument was used. Analytical data was recorded using empower work station. Analytical experiments were performed on a stainless steel column X-terra MS-C18: 100 × 3.0 mm; 5 μm with the mobile phase gradient programmed elution. A 30 °C column oven tempe-

Fig. 1. Chemical structure of aspirin, rosuvastatin, ezetimibe and clopidogrel

TABLE-1
MARKETED COMBINATION PRODUCTS

Combination products	Manufacturing company name
Aspirin 75 mg + Clopidogrel 75 mg	Blue cross, Emcure, AS pharmaceuticals, Macleods, Biocon, Aristo
Rosuvastatin 10 mg + Clopidogrel 75 mg	Macleods, Vidakem Lifesciences
Rosuvastatin 10 mg + Clopidogrel 75 mg + Aspirin 75 mg	Macleods
Rosuvastatin 10 mg + Ezetimibe 10 mg	Glenmark, Lupin, Ranbaxy

perature was used. Flow rate was kept at 1.0 mL/min, and the elution was monitored at 230 nm. Injection volume is 20.0 μ L.

Mobile phase A: Potassium dihydrogen orthophosphate (KH_2PO_4 , 6.8 g) was weighed accurately and transferred into a 1 L mobile phase preparation beaker containing 1000 mL Milli-Q-water. Mixed well with stirring, further degassed the buffer solution through 0.45 μ m filter paper using vacuum pump.

Mobile phase B: Analytical grade acetonitrile was used.

Diluent solution preparation: Mixed the mobile phase A and analytical grade acetonitrile in the ratio of 50:50 v/v. The gradient program is mentioned in Table-2.

TABLE-2
GRADIENT PROGRAM

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.01	87	13
4.0	87	13
8.0	56	44
14.0	43	57
17.0	43	57
20.0	87	13
25.0	87	13

Ezetimibe and rosuvastatin standard stock solution: Placed accurately weighed 50 mg of rosuvastatin, ezetimibe standard compounds into a 100 mL volumetric flask, diluent was added to dissolve and sonicated to reach the clear solution and made up to the volume with diluent.

Aspirin and clopidogrel standard stock solution: A 75 mg of clopidogrel and aspirin standard materials were weighed precisely and transferred into a 100 mL volumetric flask. Diluent (50 mL) was added and mixed to dissolve. Further volume was diluted with diluent.

Standard solution: Rosuvastatin-ezetimibe standard stock (1 mL) and clopidogrel-aspirin standard solutions (5 mL) were pipetted and transferred into a 50 mL volumetric flask and diluted with diluent.

Aspirin, clopidogrel and rosuvastatin test solution: Twenty tablets were weighed and made the fine powder with mortar and pestle. Equivalent to 75 mg of clopidogrel test

sample (equivalent to 75 mg aspirin, 10 mg rosuvastatin) was weighed accurately and transferred into a 100 mL class A volumetric flask. Then, 25 mL of diluent solution was added and sonicated for 15 min and made up to the volume with diluent solution and mixed well. Further, 5 mL of resulting solution was pipetted and transferred into a 50 mL volumetric flask and diluted with diluent.

Ezetimibe and rosuvastatin test solution: Twenty tablets were weighed and made the fine powder with mortar and pestle. Equivalent to 10 mg of rosuvastatin and ezetimibe test sample was weighed accurately and transferred into a 100 mL class A volumetric flask. Then, 25 mL of diluent solution was added and sonicated for 15 min and made up the volume with diluent solution and mixed well. Further, 1 mL of solution pipetted and transferred into a 50 mL volumetric flask and diluted with diluent.

System suitability limits: The % RSD for the peaks area of each component for six replicate injections of 2.0 %, peak theoretical plates should be more than 2000 and tailing factor should be not more than 2.0.

RESULTS AND DISCUSSION

Ezetimibe, rosuvastatin, aspirin and clopidogrel were well separated by using simple chromatographic conditions. Method optimization was started based on the solubility, UV absorbance and FT-IR studies.

Method development: All the four ingredients solubility studies were performed with water, acetonitrile and methanol individual solutions and mixed solutions. Aspirin, rosuvastatin, ezetimibe and clopidogrel UV spectral studies were performed. All the four compounds have maximum absorbance from 220 to 235 nm. However, 230 nm was selected for method development and validation. Fig. 2 represented the UV spectrum of four compounds.

Method development trial-1: Conditions: (a) A 3.4 g of K_2HPO_4 in 1000 mL of water used as buffer; (b) buffer and acetonitrile 80:20 v/v used as mobile phase A; (c) acetonitrile used as mobile phase B; (d) Zorbax C_{18} 150 $\times$ 4.6 mm, 5 μ column; (e) flow rate 1.0 mL/min, 30 $^\circ\text{C}$ column temperature, 230 nm;

Fig. 2. UV spectra of aspirin, rosuvastatin, ezetimibe and clopidogrel

(f) gradient program at 0 min 20 % mobile phase B at 10 min 43 %, at 15 min 83 %, at 25 min 83 %, at 28 min 20 % and at 35 min 20 %; (g) diluent: water and acetonitrile 45:55 v/v.

Observations: Elution of four compounds was aspirin at 2.8 min, rosuvastatin at 7.7 min, ezetimibe 11.7 min and clopidogrel 20.7 min. Mixed sample also injected into the HPLC system but peak shape was poor. Further method optimization can be performed with different column, mobile phase elution. Figs. 3-6 were represented the individual chromatogram of four components while Fig. 7 represented the mixed sample chromatogram.

Fig. 3. Chromatogram of aspirin

Fig. 4. Chromatogram of rosuvastatin

Fig. 5. Chromatogram of ezetimibe

Fig. 6. Chromatogram of clopidogrel

Fig. 7. Development trial chromatogram

Method development trial-2: Conditions: (a) A 6.8 g of K_2HPO_4 in 1000 mL of water used as buffer; (b) buffer and acetonitrile 80:20 v/v used as mobile phase A; (c) acetonitrile used as mobile phase B; (d) Ace C_{18} 150 $\times$ 4.6 mm, 5 μ column; (e) flow rate 1.0 mL/min, 30 $^{\circ}C$ column temperature, 230 nm; (f) gradient program at 0 min 25 % mobile phase B, at 10 min 45 %, at 15 min 80 %, at 25 min 80 %, at 28 min 25 % and at 35 min 25 %; (g) diluent: water and acetonitrile 45:55 v/v.

Observations: All the four components were separated but aspirin was eluted with unknown peak split. Fig. 8 represented the development trial chromatogram. Further optimization required get sharp peaks and no interference.

Fig. 8. Development trial chromatogram

Method development trial-3: Conditions: (a) A 6.8 g of K_2HPO_4 in 1000 mL of water used as buffer; (b) buffer used as mobile phase A; (c) acetonitrile used as mobile phase B; (d) X-Terra MS C_{18} 100×4.6 mm, 5μ column; (e) flow rate 1.0 mL/min, $30^\circ C$ column temperature, 230 nm; (f) gradient program at 0 min 13 % mobile phase B, at 4 min 13 %, at 8 min 44 %, at 14 min 57 %, at 17 min 57 %; at 20 min 13 %; and at 25 min 13 %; (g) diluent: mobile phase A and acetonitrile 50:50 v/v.

Observations: In this development trial, all the four ingredients were well separated. Aspirin eluted at 2.6 min, rosuvastatin at 9.5 min, ezetimibe at 11.0 min and clopidogrel at 17.0 min (Fig. 9). The peak tailing factor, theoretical plates results were satisfactory so this development trial conditions were finalized.

Fig. 9. Development trial chromatogram

Method validation: Method validation was performed as per ICH Q2 and USFDA guidelines. System suitability, precision, linearity, accuracy, ruggedness, robustness parameters were evaluated.

System suitability: System suitability was performed by preparing fresh standard solution and blank, placebo samples. Finalized chromatographic conditions were applied. Standard peak % area, tailing factor, theoretical plates were measured and tabulated in Table-3. Fig. 10 represented the five replicate standard stack chromatogram. The results were satisfactory *i.e.* tailing factor value is not more than 2.0; area % RSD is not more than 2.0 % and theoretical plates were above 2000.

Fig. 10. System suitability five replicate standard chromatograms

Precision: Method precision and system precision was performed with six replicate freshly prepared test solutions. Clopidogrel, rosuvastatin, aspirin tablets were used to prepare three drug test sample solutions. Rosuvastatin and ezetimibe combination tablets were used to prepare test sample solution. Six replicate test solutions were prepared and injected in to the chromatographic system. Precision results %RSD for six replicate solutions assay values were calculated and results (NMT 2.0 %) found to be satisfactory (Table-4). Figs. 11-15 represented the blank, placebo, test samples chromatograms.

Specificity: Specificity was performed by evaluating placebo and force degradation impurity peaks interference with four components. Specificity conditions are results are listed in Tables 5 and 6. Figs. 16-29 represented the overlay chromatograms with blank, placebo and force degradation studies.

Linearity: Method validation parameter linearity was performed with 50, 75, 100, 125 and 150 % linearity levels. Freshly

TABLE-3
SYSTEM SUITABILITY RESULTS

S. No.	RT (min)	Area	Tailing factor	Theoretical plates	RT (min)	Area	Tailing factor	Theoretical plates
Aspirin					Rosuvastatin			
1	2.66	4532933	1.1	7532	9.53	626631	1.3	4936
2	2.68	4526981	1.0	7562	9.53	621956	1.2	4864
3	2.70	4519637	1.2	7621	9.52	628148	1.0	4868
4	2.68	4521524	1.1	7534	9.56	628143	1.1	4876
5	2.67	4534571	1.3	7519	9.50	625831	1.2	4839
Avg.	2.68	4527129			9.53	626141		
% RSD	0.55	0.15	Avg. 1.14	Avg. 7553	0.23	0.41	Avg. 1.16	Avg. 4876
Ezetimibe					Clopidogrel			
1	11.20	259103	1.1	5163	17.12	2769953	1.3	7361
2	11.23	258169	1.3	5264	17.14	2759684	1.0	7237
3	11.25	257996	1.2	5282	17.19	2761632	1.2	7264
4	11.15	258134	1.0	5461	17.16	2764851	1.3	7315
5	11.23	258162	1.1	5348	17.31	2731589	1.1	7196
Avg.	11.21	258312			17.18	2757541		
% RSD	0.35	0.17	Avg. 1.14	Avg. 5303	0.44	0.54	Avg. 1.18	Avg. 7274

TABLE-4
METHOD PRECISION RESULTS

Active name	Precision sample preparation (% content)						RSD (%)
	1	2	3	4	5	6	
Clopidogrel, Aspirin, Rosuvastatin sample							
Aspirin	100.16	100.25	99.68	100.58	99.87	100.15	0.31
Rosuvastatin	99.88	100.21	99.79	101.21	100.13	101.31	0.67
Clopidogrel	101.25	100.12	100.61	99.76	99.86	100.56	0.56
Rosuvastatin and Ezetimibe sample							
Rosuvastatin	101.20	100.10	100.31	101.02	100.54	100.26	0.44
Ezetimibe	100.13	99.99	100.15	100.9	101.58	100.37	0.65

Fig. 11. Blank chromatogram

Fig. 12. Clopidogrel, rosuvastatin, aspirin tablets placebo chromatogram

Fig. 13. Ezetimibe and rosuvastatin tablets placebo chromatogram

Fig. 14. Aspirin, rosuvastatin, clopidogrel test sample chromatogram

Fig. 15. Ezetimibe and rosuvastatin test sample chromatogram

Fig. 16. Blank and standard overlay chromatogram

Fig. 17. Placebo and standard overlay chromatogram

TABLE-5
SPECIFICITY CONDITIONS AND RESULTS

Name of stress and condition	Degradation (%)	Purity angle	Purity threshold
Clopidogrel, Aspirin, Rosuvastatin test sample			
Acid stress/1N-60 °C/90 min	6.9	0.073	0.255
Base stress/1N-60 °C/2 h	1.7	0.071	0.309
Peroxide stress/3 %- 0 °C/1 h	10.9	0.146	0.283
Water stress-70 °C/2 h	1.7	0.084	0.243
Thermal (80 °C for 6 h)	13.7	0.094	0.255
UV energy of 200-watt h ² /m	—	—	—
Rosuvastatin and Ezetimibe test sample			
Acid stress/1N-60 °C/90 min	6.9	0.073	0.255
Base stress/1N-60 °C/2 h	1.7	0.071	0.309
Peroxide stress/3 %- 50 °C/1 h	10.9	0.146	0.283
Water stress-70 °C/2 h	1.7	0.084	0.243
Thermal (80 °C for 6 h)	13.7	0.094	0.255
UV energy of 200-watt h ² /m	—	—	—

TABLE-6
SPECIFICITY IMPURITY RESULTS

Stress condition	Peak-1		Peak-2		Peak-3	
	RT (min)	Area (%)	RT (min)	Area (%)	RT (min)	Area (%)
Clopidogrel, aspirin, rosuvastatin test sample						
Acid	6.547	6.1	14.566	4.6	20.866	2.9
Base	6.576	6.3	14.564	4.8	ND	ND
Peroxide	6.573	5.9	ND	ND	20.863	2.8
Thermal	ND	ND	14.569	4.6	20.869	30
UV	6.533	6.0	14.567	4.9	ND	ND
Water	6.566	5.9	14.547	4.4	ND	ND
Rosuvastatin and Ezetimibe test sample						
Acid	6.503	6.2	14.543	5.1	20.836	2.8
Base	6.506	5.8	14.563	4.9	ND	ND
Peroxide	6.543	6.3	ND	ND	ND	ND
Thermal	ND	ND	14.561	4.9	20.800	2.8
UV	ND	ND	ND	ND	20.807	2.7
Water	ND	ND	14.560	5.0	20.862	3.0

Fig. 18. Aspirin, rosuvastatin, clopidogrel acid degradation chromatogram

Fig. 22. Aspirin, rosuvastatin, clopidogrel UV degradation chromatogram

Fig. 19. Aspirin, rosuvastatin, clopidogrel base degradation chromatogram

Fig. 23. Aspirin, rosuvastatin, clopidogrel water degradation chromatogram

Fig. 20. Aspirin, rosuvastatin, clopidogrel peroxide degradation chromatogram

Fig. 24. Ezetimibe and rosuvastatin acid degradation chromatogram

Fig. 21. Aspirin, rosuvastatin, clopidogrel thermal degradation chromatogram

Fig. 25. Ezetimibe and rosuvastatin base degradation chromatogram

Fig. 26. Ezetimibe and rosuvastatin peroxide degradation chromatogram

Fig. 28. Ezetimibe and rosuvastatin UV degradation chromatogram

Fig. 27. Ezetimibe and rosuvastatin thermal degradation chromatogram

Fig. 29. Ezetimibe and rosuvastatin water degradation chromatogram

prepared standard stock solution was used to prepare all linearity levels. All linearity levels were injected into the chromatographic system. Correlation coefficient value was calculated and

found to be satisfactory *i.e.* above 0.999 for all components. Linearity graph was plotted with concentration and area values and the linearity results are shown in Table-7.

TABLE-7
LINEARITY RESULTS

Linearity level	Aspirin		Rosuvastatin		Ezetimibe		Clopidogrel	
	Conc. (ppm)	Area	Conc. (ppm)	Area	Conc. (ppm)	Area	Conc. (ppm)	Area
50 %	37.50	2428617	5.0	317151	5.0	181457	37.50	1378126
75 %	56.25	3392659	7.5	475190	7.5	268441	56.25	2075059
100 %	75.00	4532944	10.00	626634	10.0	259104	75.00	2769956
125 %	93.75	6026301	12.5	799351	12.5	403988	93.75	3472472
150 %	112.5	7604519	15.00	966915	15.0	780040	112.5	4168790
C.C.	0.99921		0.9997		0.9991		0.9999	

TABLE-8
ACCURACY RESULTS

Clopidogrel, Aspirin and Rosuvastatin Accuracy results									
Level	Qty. added (ppm)	Qty. recovered (ppm)	Recovery (%)	Qty. added (ppm)	Qty. recovered (ppm)	Recovery (%)	Qty. added (ppm)	Qty. recovered (ppm)	Recovery (%)
	Aspirin			Rosuvastatin			Clopidogrel		
50 %	37.52	37.49	99.92	5.01	5.05	100.80	5.09	5.12	100.59
	37.51	37.53	100.05	5.03	5.04	100.20	5.04	5.06	100.40
	37.52	37.54	100.05	5.02	5.03	100.20	5.03	5.05	100.40
100 %	75.01	75.06	100.07	10.10	10.14	100.40	10.04	10.06	100.20
	75.06	75.03	99.96	10.13	10.11	99.80	10.06	10.09	100.30
	75.04	75.07	100.04	10.09	10.10	100.10	10.08	10.02	99.40
150 %	112.50	112.53	100.03	15.03	15.10	100.47	15.02	15.09	100.47
	112.53	112.55	100.02	15.04	15.10	100.40	15.04	15.07	100.20
	112.51	112.57	100.05	15.01	15.08	100.47	15.03	15.04	100.07
Rosuvastatin and ezetimibe test sample									
	Rosuvastatin			Ezetimibe					
50 %	5.02	5.06	100.80	37.50	37.53	100.08			
	5.04	5.07	100.60	37.54	37.50	99.89			
	5.01	5.06	101.00	37.55	37.54	99.97			
100 %	10.11	10.15	100.40	75.06	75.09	100.04			
	10.10	10.13	100.30	75.03	75.04	100.01			
	10.08	10.10	100.20	75.08	75.01	99.91			
150 %	15.05	15.11	100.40	112.51	112.56	100.04			
	15.04	15.07	100.20	112.55	112.50	99.96			
	15.06	15.03	99.80	112.54	112.58	100.04			

Accuracy: Accuracy was performed with three different concentration levels. A 50, 100 and 150 % accuracy levels were conducted. Placebo stock solutions were spiked to API stock solutions. Clopidogrel, aspirin, rosuvastatin samples were prepared separately and rosuvastatin and ezetimibe accuracy samples were prepared separately. Accuracy results were calculated and results % recovery was satisfactory. Percentage recovery result was found to be between 97 % to 103 %. Table-8 shown the accuracy levels for clopidogrel, aspirin and rosuvastatin combination products and rosuvastatin and ezetimibe combination.

Ruggedness: Ruggedness was performed for solution stability store conditions at room temperature and refrigerator. Mobile phase stability evaluated at room temperature. Initially prepared two samples (retained from precision analysis) were kept at room temperature and refrigerator and conducted the study at day-1 (24 h) and day-3 (72 h). Mobile phase stability study evaluated at day-1 (24 h) and day-3 (72 h). Tables 9 and 10 represented the ruggedness results.

Robustness: Robustness was performed for mobile phase flow rate variation, column oven temperature variation and filter validation. All the variations results (Tables 11 and 12) were found to be satisfactory.

Conclusion

A simple HPLC method was developed for the quantification of four drug components in solid dosage forms. Aspirin, clopidogrel, rosuvastatin and ezetimibe were quantified with

Duration	Sample solution-1		Sample solution-2	
	Actual	Variation (%)	Actual	Variation (%)
Aspirin				
Initial	101.54	NA	100.56	NA
Day-1	100.92	0.6	100.32	0.2
Day-3	100.26	1.3	100.34	0.2
Rosuvastatin (clopidogrel, aspirin, rosuvastatin tablets)				
Initial	100.68	NA	99.98	NA
Day-1	99.89	0.79	101.00	1.02
Day-3	101.60	0.92	100.06	0.08
Rosuvastatin (rosuvastatin and ezetimibe tablets)				
Initial	100.14	NA	100.65	NA
Day-1	99.98	0.16	100.31	0.34
Day-3	101.00	0.86	99.87	0.78
Ezetimibe				
Initial	100.14	NA	100.65	NA
Day-1	99.98	0.16	100.31	0.34
Day-3	101.00	0.86	99.87	0.78
Clopidogrel				
Initial	100.14	NA	100.65	NA
Day-1	99.98	0.16	100.31	0.34
Day-3	101.00	0.86	99.87	0.78

stability indicating HPLC method. Method validation was performed as per ICH Q2, USFDA guidance documents. Precision results % of assay and %RSD values was within in the limit. Linearity correlation coefficient above 0.999 for all the four

Duration	Clopidogrel, aspirin, rosuvastatin tablets						Rosuvastatin, Ezetimibe tablets			
	Aspirin		Rosuvastatin		Clopidogrel		Rosuvastatin		Ezetimibe	
	Tailing factor	RSD (%)	Tailing factor	RSD (%)	Tailing factor	RSD (%)	Tailing factor	RSD (%)	Tailing factor	RSD (%)
Initial	1.2	0.59	1.3	0.61	1.4	0.61	1.4	0.61	1.2	0.71
Day-1	1.4	0.61	1.1	0.57	1.2	0.54	1.2	0.57	1.5	0.59
Day-3	1.1	0.54	1.4	0.61	1.6	0.58	1.6	0.53	1.4	0.64

Parameter	Aspirin			Rosuvastatin (clopidogrel combination)		Rosuvastatin (ezetimibe combination)		Clopidogrel		Ezetimibe	
	Tailing factor	%RSD (5 inj.)		Tailing factor	%RSD (5 inj.)	Tailing factor	%RSD (5 inj.)	Tailing factor	%RSD (5 inj.)	Tailing factor	%RSD (5 inj.)
Flow rate (mL/min)	0.8	1.0	0.63	1.1	0.45	1.1	0.61	1.2	0.59	1.3	0.60
	1.2	0.9	0.59	1.0	0.16	1.0	0.54	1.1	0.68	1.6	0.63
Temp. (°C)	25	1.1	0.14	1.2	0.61	0.9	0.16	1.4	0.61	1.3	0.54
	35	1.0	1.0	0.9	0.56	1.1	1.13	1.3	0.70	1.2	0.59

Standard solution	Aspirin		Rosuvastatin (clopidogrel combination)		Rosuvastatin (ezetimibe combination)		Clopidogrel		Ezetimibe	
	% Assay (w/w)	Difference (%)	% Assay (w/w)	Difference (%)	% Assay (w/w)	Difference (%)	% Assay (w/w)	Difference (%)	% Assay (w/w)	Difference (%)
Centrifuged 0.45 µm PVDF filter	100.80	NA	103.16	NA	100.77	NA	101.30	NA	100.61	NA
	100.63	0.17	101.15	2.01	100.10	0.67	100.25	1.05	99.81	0.80

components was observed. Specificity confirmed no interference with placebo, diluent and unknown impurity peaks. Ruggedness and robustness results confirmed the HPLC method compatibility in different systems and laboratories. Eventually, this HPLC method can be applied for regular medicinal product analysis.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. N. Khalaf, C. Yuan, T. Hamada, Y. Cao, A. Babic, V. Morales-Oyarvide, P. Kraft, K. Ng, E. Giovannucci, S. Ogino, M. Stampfer, B.B. Cochrane, J.E. Manson, C.B. Clish, A.T. Chan, C.S. Fuchs and B.M. Wolpin, *Gastroenterology*, **154**, 1380 (2018); <https://doi.org/10.1053/j.gastro.2017.12.001>.
2. R. Fernandez-Jimenez, J. Latina, R. Hajjar, V. Fuster, T. Wang and W. Blot, *J. Am. Coll. Cardiol.*, **71**, A1892 (2018); [https://doi.org/10.1016/S0735-1097\(18\)32433-1](https://doi.org/10.1016/S0735-1097(18)32433-1).
3. K. Vyshnavi, M. Bhashetty, N. Swain, M. Venkataswamy and A. Ramesh, *Res. J. Pharm. Dos. Forms Technol.*, **9**, 45 (2017); <https://doi.org/10.5958/0975-4377.2017.00009.X>.
4. T. Zhu, B. Parker, T. Wojtkowski, T. Nishimura, J.P. Garg, D. Han, O. Fisniku and J. Keirns, *Clin. Pharmacokinet.*, **56**, 747 (2017); <https://doi.org/10.1007/s40262-016-0474-4>.
5. A.V. Khera, O.V. Demler, S.J. Adelman, H.L. Collins, R.J. Glynn, P.M. Ridker, D.J. Rader and S. Mora, *Circulation*, **135**, 2494 (2017); <https://doi.org/10.1161/CIRCULATIONAHA.116.025678>.
6. S. Takase and T. Matoba, *Arterioscler. Thromb. Vasc. Biol.*, **37**, e54 (2017); <https://doi.org/10.1161/ATVBAHA.117.309301>.
7. E.T. Kato, C.P. Cannon, M.A. Blazing, E. Bohula, S. Guneri, J.A. White, S.A. Murphy, J.G. Park, E. Braunwald and R.P. Giugliano, *J. Am. Heart Assoc.*, **6**, e006901 (2017); <https://doi.org/10.1161/JAHA.117.006901>.
8. J. Schmucker, H. Wienbergen, A. Fach, L.M. Marin, D. Garstka, J. Stehmeier, E. Fiehn and R. Hambrecht, *Eur. Heart J.*, **38**(Suppl. 1), ehx501.263 (2017); <https://doi.org/10.1093/eurheartj/ehx501.263>.
9. T.K. Bergmann, K. Agergaard, M. Mau-Sørensen, T.B. Stage, T.L. Jørgensen, R.E. Hassel, K.D. Steffensen, J.W. Pedersen, M.L. Milo, S.H. Poulsen, A. Pottegård, J. Hallas and K. Brosen, *Clin. Ther.*, **39**, e61 (2017); <https://doi.org/10.1016/j.clinthera.2017.05.189>.
10. H. Kahma, A.M. Filppula, M. Neuvonen, E.K. Tarkiainen, A. Tornio, M.T. Holmberg, M.K. Itkonen, M. Finel, P.J. Neuvonen, M. Niemi and J.T. Backman, *Drug Metab. Dispos.*, **46**, 141 (2018); <https://doi.org/10.1124/dmd.117.078162>.
11. A. Bhadoriya, M. Sanyal, P.A. Shah and P.S. Shrivastav, *Biomed. Chromatogr.*, **32**, 4291 (2018); <https://doi.org/10.1002/bmc.4291>.
12. H. Kim, H.Y. Choi, Y.H. Kim, K.S. Bae, J. Jung, H. Son and H.S. Lim, *Drug Des. Devel. Ther.*, **12**, 815 (2018); <https://doi.org/10.2147/DDDT.S158408>.
13. D. Sireesha, M.L. Monika and V. Bakshi, *Asian J. Pharm. Anal.*, **7**, 135 (2017); <https://doi.org/10.5958/2231-5675.2017.00021.7>.
14. J. Yadav and A. Sharma, *Pharma Tutor*, **5**, 35 (2017).
15. A.S. Sankar, P. Shanmugasundaram and V. Ravichandiran, *Curr. Anal. Chem.*, **13**, 386 (2017); <https://doi.org/10.2174/1573411013666170127163707>.