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Development of RP-HPLC method for simultaneous estimation of aspirin, clopidogrel and rosuvastatin in tablet dosage form

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Abstract
Objective: The scope of present work is to expand and develop RP-HPLC method for estimation of Aspirn, Clopisdogrel and Rosuvastatin Tablet.
Methods: The method was preferred with various columns like C18 column, koromasil, Hypersil BDS. Among them C18 was found to be ideal as it has good peak shape 1ml/min flow rate and a proper mobile phase was developed to obtain ideal and sharp peaks with optimum resolution.
Results: The system suitability parameters were evaluated from standard chromatogram and compared with sample chromatogram of aspirin, clopidogrel, and rosuvastatin. Also there peak areas and retention time were observed to obtain an ideal peak. Several mobile trials were performed and at 14th trial a proper chromatogram was generated with ideal peak obtained for all the three dugs similar to that of standard.
Conclusion: The retention times for Aspirin, Clopidogrel, and Rosuvastatin were 8.933, 4.953, and 6.783 respectively and pure peaks were obtained. Therefore a new method was developed for estimation of aspirin, clopidogrel and rosuvastatin us in RP-HPLC method.

Keywords: aspirin, clopidogrel, rosuvastatin, development, RP-HPLC method, mobile trials, retention times

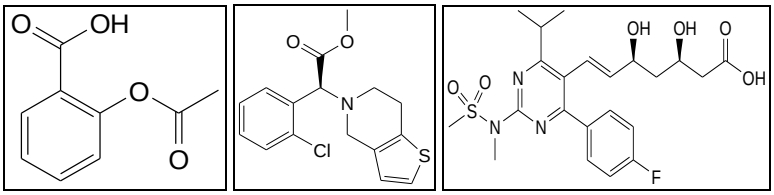
1. Introduction

1.1 Drug Profile

Aspirin, acetylsalicylic acid (NSAIDS), is a medication used to treat pain, fever and inflammation. It is used in pericarditis, and rheumatic fever. Aspirin given after a heart attack decreases the risk of death.

Clopidogrel is used as medication to reduce the risk of heart disease, and stroke. It is also used together with aspirin in heart attacks and following the placement of a coronary artery stent(dual antiplatelet therapy).

Rosuvastatin is a member of the drug class of statins used to treat high cholesterol and related conditions. Putative beneficial effects of rosuvastatin therapy on chronic heart failure may be negated by increase in collagen turnover markers as well as reduction in plasma coenzymeQ10 levels in patients with chronic heart failure. The combination of three drugs reduced frequency of dosing and to reduce related adverse effect and side effect of individual drug as compare to monotherapy.



Aspirin Clopidogrel Rosuvastatin

Structure of Aspirin, Clopidogrel and Rosuvastatin

1.2 Analytical method development [1-3]

Pharmaceutical products formulated with more than one drug, typically referred to as combination products. These combination products can present daunting challenges to the analytical chemist responsible for the development and validation of analytical methods. The development and validation of analytical methods [Spectrophotometric, High performance liquid chromatography (HPLC) & High performance thin layer chromatography (HPTLC)] for drug products containing more than one active ingredient. The official test methods that result from these processes are used by quality control laboratories to ensure the identity, purity, potency, and performance of drug products.

The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing ones. Very often there

is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (resulting in their withdrawal from the market), development of patient resistance and introduction of better drugs by competitors. Under these conditions, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs.

2. Experimental Work

2.1 Materials [18-19]

Method development and validation for estimation of Aspirin, Clopidogrel and Rosuvastatin as active pharmaceutical ingredients and tablet forms following materials are use

Table 1: Detail of instruments used

SR. No.	Instruments	Specification and Manufacturer
1	Double Beam UV-Visible Spectrophotometer	VU-Visible 1700, Shimadzu Limited
2	HPLC	SPD-20AT, Shimadzu Limited
3	Analytical balance	AX-200, Precisa Limited
4	pH Meter	Chemiline, India
5	Ultra Sonic Cleaner	Toshcon, Toshniwal Process Instrument Pvt. Ltd., Ajmer

Table 2: System Specification of HPLC (SPD-20AT)

Sr. No	Name	Specification
1	Pump	LC-20AT
2	Detector	SPD-20AT
3	Column	BDS Hypersil C18, 250mm X 4.6 mm, 5µ(particle size), Thermo scientific
4	Injector	Rheodyne injector (20µl Capacity)
5	Syringe	Hamilton (25µl)
6	Chromatographic Software	Spinchrom

Table 3: Detail of chemicals used

Sr. No	Chemicals	Grade	Manufactirer
1	Acetonitrile	HPLC	Merck, Rankem
2	Potassium Dihydrogen Phosphate	AR	Merck, Rankem
3	Water	HPLC	HPLC Grade
4	Orthophosphoric Acid	AR	Merck, Rankem
5	Tri Ethyl Amine	AR	Merck, Rankem
6	Methanol	HPLC	Merck, Rankem

Table 4: Detail of commercial formulations used

Drug	Composition	Company name	Brand
Aspirin	15% w/w	Glenmark	Supirocin-B plus
Clopidogrel	15% w/w		
Rosuvastatin	2%w/w		

2.2 Methods

In the present research work RP-HPLC methods have been developed for the estimation of Aspirin, Clopidogrel, and Rosuvastatin in tablet formulations.

2.2.1 Preparation of Solutions

1. **Preparation of standard stock solution of aspirin:** Take 10 mg of aspirin into a 10 ml volumetric flask and dilute with methanol.
2. **Preparation of working stock solution of aspirin:** From the above standard solution 0.15 ml is taken and diluted to 10 ml to give a concentration of 15 mcg/ml of standard solution of Aspirin.
3. **Preparation of standard stock solution of clopidogrel:** Take10 mg of Clopidogrelinto a 10 ml Volumetric flask and dilute with methanol,
4. **Preparation of working stock solution of clopidogrel:** From the above standard solution 0.15 ml is taken and diluted to 10 ml to get a concentration of 15 mcg/ml of standard solution of Clopidogrel.
5. **Preparation of standard stock solution of rosuvastatin:** Take 10 mg of Rosuvastatin into a 10 ml volumetric flask and dilute with methanol. From above solution take 0.2 ml and dilute with 10 ml of methanol to get 20 mcg/ml a concentration.
6. **Preparation of working stock solution of rosuvastatin:** From the above standard solution 1 ml is taken and diluted to 10 ml to get a concentration of 2 mcg/ml of standard solution of Rosuvastatin.
7. **Preparation of standard stock solution (combined aspirin, clopidogrel, and rosuvastatin):** Take 1 ml from Aspirin stock solution and 1 ml from Clopidogrel stock solution and 1 ml Rosuvastatin stock solution dilute with mobile phase(mobile phase which used for trials) to get a concentration of Aspirin15 mcg/ml, Clopidogrel 15 mcg/ml and Rosuvastatin 2 mcg/ml.
8. **Preparation of sample stock solution (from commercial formulation):** Transfer about 15 mg tablet powder into a 100 ml volumetric flask. Make up to 100 ml methanol and shake for 15 minutes. Put on water bath at 60° C for 10 minutes. Make up volume with methanol. Filter the solution with what man filter paper and get a

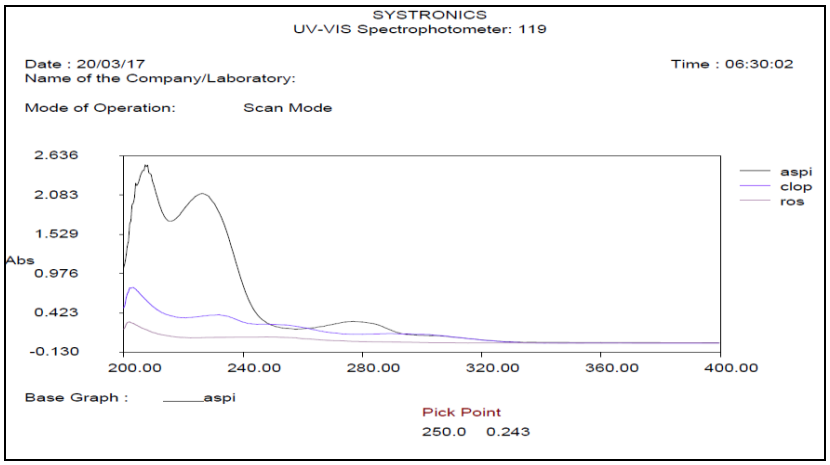
Concentration Aspirin 15 mcg/ml, Clopidogrel 15 mcg/ml and Rosuvastatin 2 mcg/ml.

Take 1 ml from above sample stock solution and make up volume 10 ml. and get a Concentration Aspirin (15mcg/ml), Clopidogrel 15(mcg/ml), and Rosuvastatin 2mcg/ml

9. Preparation of Mobile Phase

Take about 6.8 gm of potassium dihydrogen ortho phosphate reagent into a 1000 ml beaker. Add 800 ml water and dissolve it. Adjust pH 3.5 of the solution with 1% ortho phosphoric acid. Make up volume upto 1000 ml with water.(buffer solution ph 3.5)

2.2.2 Determination of absorbance maxima (λ max) of aspirin, clopidogrel, and rosuvastatin



Procedure for wavelength selection

- Prepare Aspirin 15mcg/ml, Clopidogrel 15mcg/ml, and Rosuvastatin 2mcg/ml respectively in methanole.
- Run all the spectra individually between 190 nm to 370 nm and overlap them.
- Select suitable wave length (select isoabsorptive point, if observed any).
- In this case, there is no is oabsorptive point.
- At 213 nm, we would almost some absorbances.

So final wavelength should be 213 nm for HPLC analysis

2.2.3 Optimization of mobile phase, column and solvent system

Sr. Trial	Mobile phase	Column	Injection volume (microliter)	Column temp	Flow rate(ml/min)	Observation
1	Water : Methanol (50:50)	BDS Hypersil C $_{18}$,250 mm X 4.6 mm, 5 µ(partical size),Thermo scientific	20	25	1ml/min	One peak observed
2	Water : Methanol (50:50)	BDS Hypersil C $_{18}$,250 mm X 4.6 mm, 5 µ(partical size),Thermo scientific	20	25	1ml/min	Only clopidogrel peak observed
3	Water : Methanol (50:50)	BDS Hypersil C $_{18}$,250 mm X 4.6 mm, 5 µ(partical size),Thermo scientific	20	25	1ml/min	Only aspirin peak observed
4	Water : Methanol (20:80)	BDS Hypersil C $_{18}$,250 mm X 4.6 mm, 5 µ(partical size),Thermo scientific	20	25	1ml/min	Two peak observed
5	Water : Acetonitrile (50:50)	BDS Hypersil C $_{18}$,250 mm X 4.6 mm, 5 µ(partical size),Thermo scientific	20	25	1ml/min	two peak observed
6	Water : Acetonitrile (30:70)	BDS Hypersil C $_{18}$,250 mm X 4.6 mm, 5 µ(partical size),	20	25	1ml/min	Two peak observed
7	Buffer pH(5.5) : Methanol (50:50)	BDS Hypersil C $_{18}$,250 mm X 4.6 mm, 5 µ(partical size),Thermo scientific	20	25	1ml/min	two peak observed
8	Buffer pH(4.5) : Methanol (50:50)	BDS Hypersil C $_{18}$,250 mm X 4.6 mm, 5 µ(partical size),Thermo	20	25	1ml/min	Only Rosuvastatin peak observed

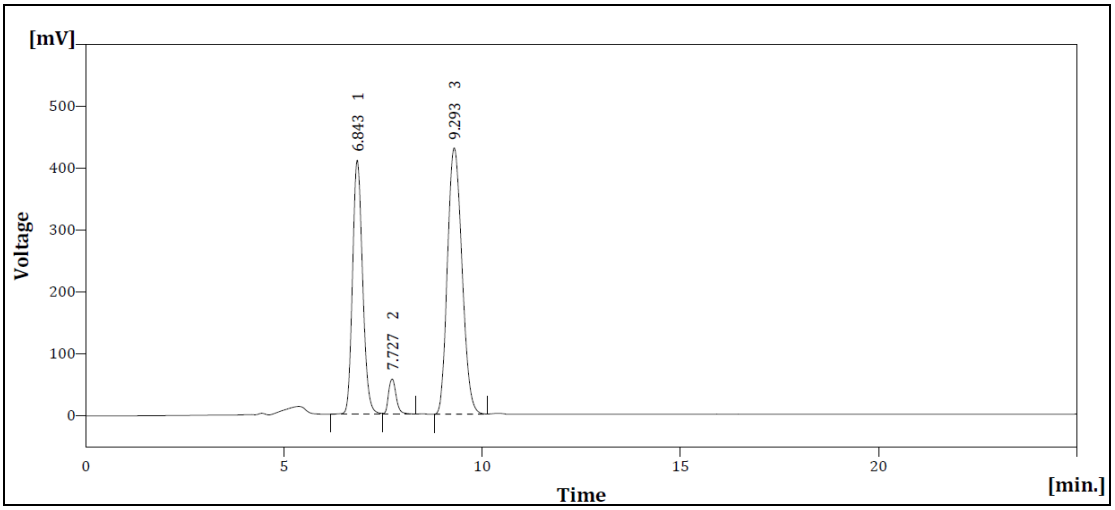
Trail 9

Mobile phase Buffer ph.(4.5): Methanol (50:50)

Conditions

- Column: BDS Hypersil C $_{18}$, 250 mm X 4.6 mm, 5 µ(partical size),Thermo scientific
- Injection volume : 20 µl
- Column temperature: 25^{0}C
- Sample temperature: 25^{0}C
- Flow rate : 1 ml/min
- λ max: 213 nm

Observation: Though all the peak were reported but. the time interval between the peaks was less



Observation: Though all the peak were reported but the time interval between the peaks was less

Result Table (Uncal - C:\Documents and Settings\My-Pc\Desktop\WORK1\DATA\clp-ros-asp buffer (pH 4.5)-methanol 50-50)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]
1	6.843	7255.201	410.481	38.218	45.7
2	7.727	758.635	56.635	3.996	6.3
3	9.293	10970.125	430.310	57.786	47.9
	Total	18983.961	897.426	100.000	100.0

Column Performance Table (From 50% - C:\Documents and Settings\My-Pc\Desktop\WORK1\DATA\clp-ros-asp buffer (pH 4.5)-methanol 50-50)

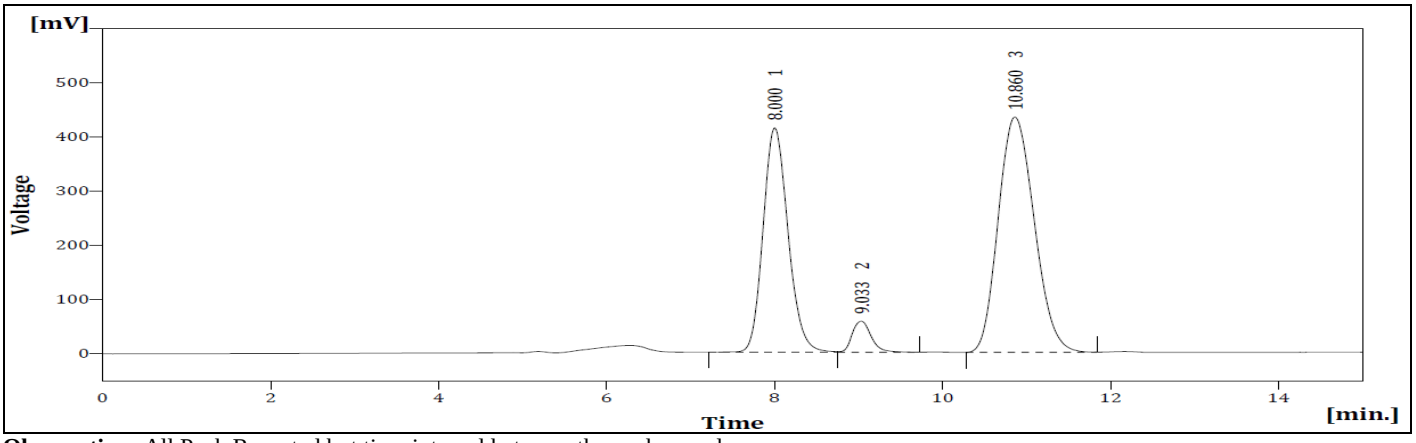
	Reten. Time	Asymmetry [-]	Efficiency [th.pl]	Resolution [-]
1	6.843	1.284	3473	-
2	7.727	1.286	7744	2.166
3	9.293	1.274	2893	3.006

Trail 10

Mobile phase: Buffer ph.(4.5): Methanol (60:40)

Conditions:

- Column: BDS Hypersil C $_{18}$, 250 mm X 4.6 mm, 5 µ(partical size),Thermo scientific
- Injection volume : 20 µl
- Column temperature: 25^{0}C
- Sample temperature: 25^{0}C
- Flow rate : 1 ml/min
- λ max: 213 nm



Observation: All Peak Reported but time interval between the peaks was less

Result Table (Uncal - C:\Documents and Settings\My-Pc\Desktop\WORK1\DATA\clp-ros-asp buffer (pH 4.5)-methanol 60-40)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]
1	8.000	8556.751	414.248	38.218	45.7
2	9.033	894.587	57.150	3.996	6.3
3	10.860	12937.914	434.291	57.786	48.0
	Total	22389.252	905.689	100.000	100.0

Column Performance Table (From 50% - C:\Documents and Settings\My-Pc\Desktop\WORK1\DATA\clp-ros-asp buffer (pH 4.5)-methanol 60-40)

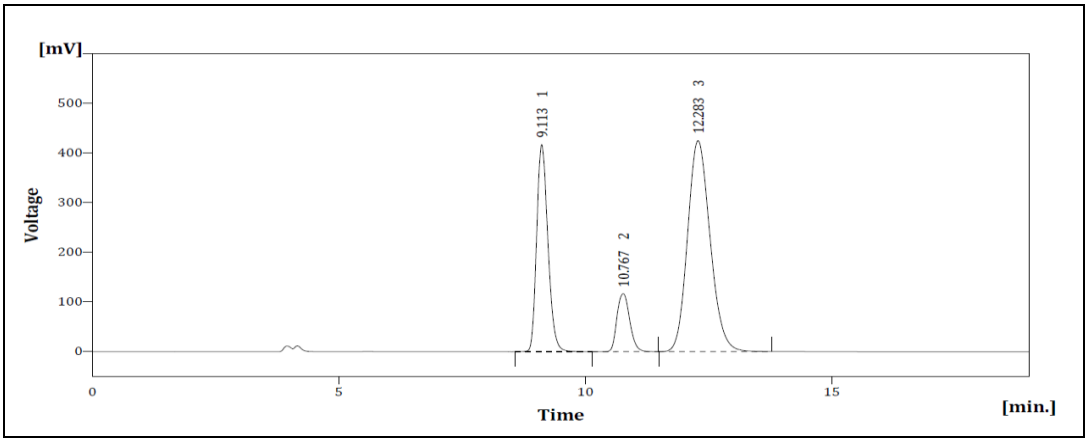
	Reten. Time	Asymmetry [-]	Efficiency [th.pl]	Resolution [-]
1	8.000	1.278	3463	-
2	9.033	1.281	7635	2.159
3	10.860	1.291	2916	3.000

Trail 11

Mobile Phase: Buffer ph. (3.5): Methanol (70:30)

Conditions:

- Column: BDS Hypersil C $_{18}$, 250 mm X 4.6 mm, 5 µ(partical size),Thermo scientific
- Injection volume : 20 µl
- Column temperature: 25^{0}C
- Sample temperature: 25^{0}C
- Flow rate : 1 ml/min
- λ max: 213 nm



Observation: All Peak Reported but time interval between the peaks was less

Result Table (Uncal - C:\Documents and Settings\My-Pc\Desktop\WORK1\DATA\clp-ros-asp buffer (pH 3.5)-methanol 70-30)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]
1	9.113	6640.967	417.603	29.835	43.5
2	10.767	2098.654	116.911	9.428	12.2
3	12.283	13519.640	425.293	60.737	44.3
	Total	22259.262	959.807	100.000	100.0

Column Performance Table (From 50% - C:\Documents and Settings\My-Pc\Desktop\WORK1\DATA\clp-ros-asp buffer (pH 3.5)-methanol 70-30)

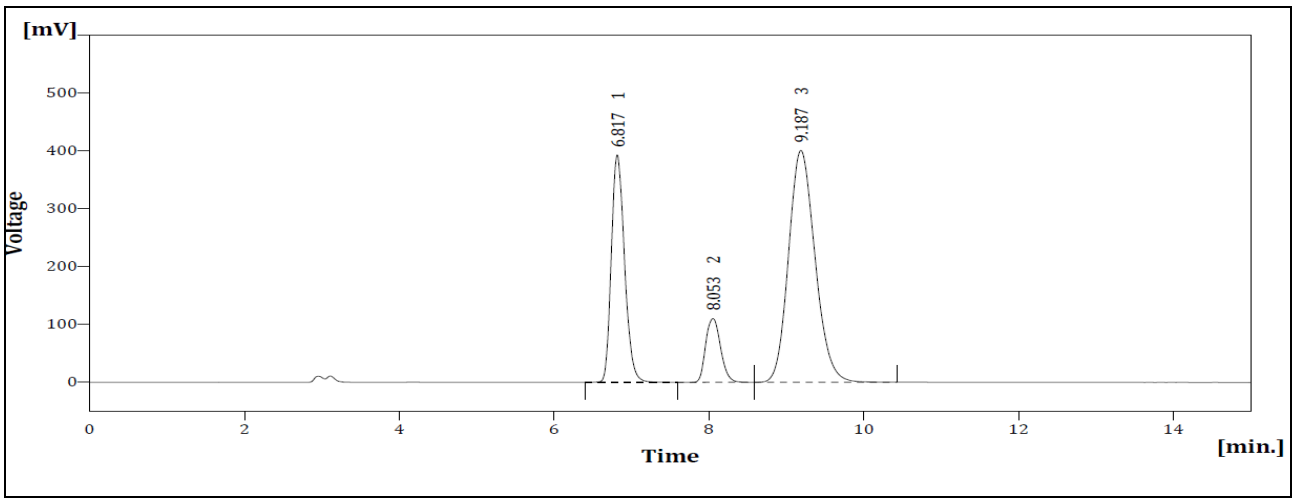
	Reten. Time	Asymmetry [-]	Efficiency [th.pl]	Resolution [-]
1	9.113	1.379	7562	-
2	10.767	1.242	7815	3.648
3	12.283	1.270	3434	2.288

Trail 12

Mobile phase: Buffer ph.(3.5): Methanol (50:50)

Conditions:

- Column: BDS Hypersil C $_{18}$, 250 mm X 4.6 mm, 5 µ(partical size),Thermo scientific
- Injection volume : 20 µl
- Column temperature: 25^{0}C
- Sample temperature: 25^{0}C
- Flow rate : 1 ml/min
- λmax:213nm



Observation: All Peak Reported but poor peak obtained.

Result Table (Uncal - C:\Documents and Settings\My-Pc\Desktop\WORK1\DATA\clp-ros-asp buffer (pH 3.5)-methanol 50-50)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]
1	6.817	4684.123	393.772	29.830	43.5
2	8.053	1480.225	110.259	9.426	12.2
3	9.187	9538.566	401.119	60.744	44.3
	Total	15702.914	905.150	100.000	100.0

Column Performance Table (From 50% - C:\Documents and Settings\My-Pc\Desktop\WORK1\DATA\clp-ros-asp buffer (pH 3.5)-methanol 50-50)

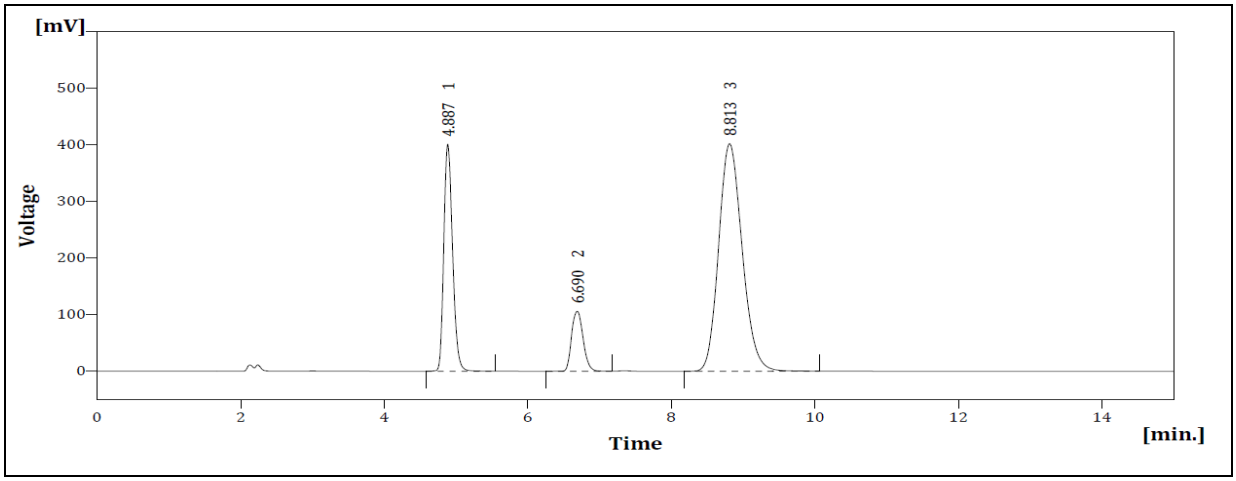
	Reten. Time	Asymmetry [-]	Efficiency [th.pl]	Resolution [-]
1	6.817	1.395	7388	-
2	8.053	1.240	7895	3.638
3	9.187	1.275	3478	2.300

Trail 13

Mobile phase: Buffer pH.(3.0): Methanol (50:50)

Conditions:

- Column: BDS Hypersil C $_{18}$, 250 mm X 4.6 mm, 5 µ(partical size),Thermo scientific
- Injection volume : 20 µl
- Column temperature: 25^{0}C
- Sample temperature: 25^{0}C
- Flow rate : 1 ml/min
- λ max: 213 nm



Observation: All Peak Reported but poor peaks were obtained

Result Table (Uncal - C:\Documents and Settings\My-Pc\Desktop\WORK1\DATA\clp-ros-asp buffer (pH 3.0)-methanol 50-50)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]
1	4.887	3409.928	401.468	24.783	44.1
2	6.690	1183.311	105.992	8.600	11.7
3	8.813	9166.180	402.223	66.617	44.2
	Total	13759.420	909.682	100.000	100.0

Column Performance Table (From 50% - C:\Documents and Settings\My-Pc\Desktop\WORK1\DATA\clp-ros-asp buffer (pH 3.0)-methanol 50-50)

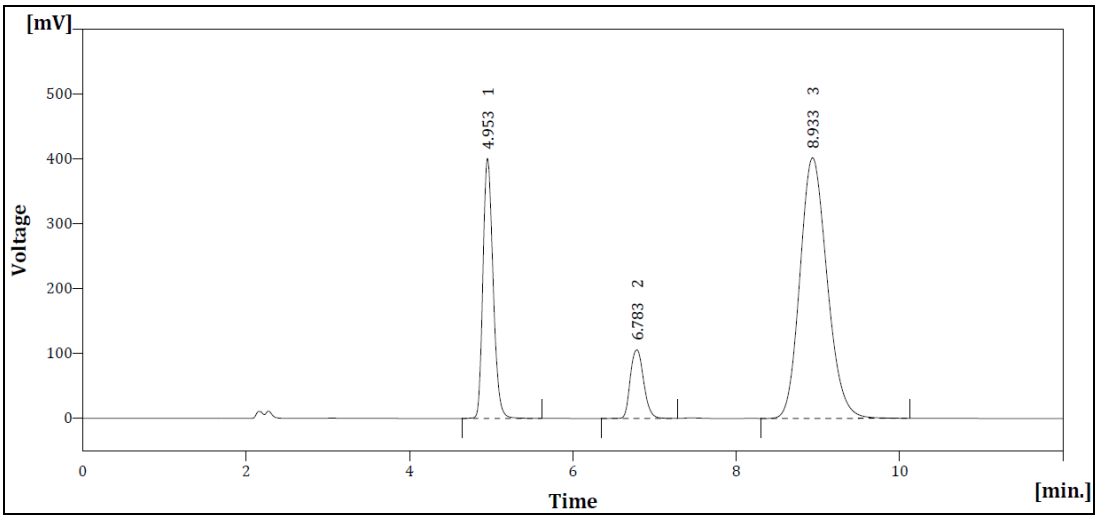
	Reten. Time	Asymmetry [-]	Efficiency [th.pl]	Resolution [-]
1	4.887	1.355	7441	-
2	6.690	1.214	7653	6.773
3	8.813	1.261	3447	4.685

Trail 14

Mobile phase: Buffer pH.(3.0): Methanol (40:60)

Conditions:

- Column: BDS Hypersil C $_{18}$, 250 mm X 4.6 mm, 5 µ(partical size),Thermo scientific
- Injection volume : 20 µl
- Column temperature: 25^{0}C
- Sample temperature: 25^{0}C
- Flow rate : 1 ml/min
- λ max: 213 nm



For optimization of mobile phase, column and solvent system several trails were taken for the simultaneous estimation of Aspirin, Clopidogrel and Rosuvastatin by HPLC.

Result Table (Uncal - C:\Documents and Settings\My-Pc\Desktop\WORK1\DATA\clp-ros-asp std)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]
1	4.953	3456.303	401.266	24.783	44.1
2	6.783	1199.440	105.974	8.600	11.7
3	8.933	9290.481	402.239	66.616	44.2
	Total	13946.224	909.478	100.000	100.0

Column Performance Table (From 50% - C:\Documents and Settings\My-Pc\Desktop\WORK1\DATA\clp-ros-asp std)

	Reten. Time	Asymmetry [-]	Efficiency [th.pl]	Resolution [-]
1	4.953	1.419	7646	-
2	6.783	1.238	7868	6.873
3	8.933	1.284	3411	4.686

3. Summary and Conclusion

The analytical methods were attempted to develop for the Simultaneous estimation of Aspirin, Clopidogrel, and Rosuvastatin tablet by RP-HPLC. The trial 14 was found to be accurate and reliable comparative to remaining trials. This trial 14 was used for validation. Mobile phase used was buffer pH(3): methanol (40:60), retention time for Aspirin, Clopidogrel and Rosuvastatin were found to be 8.933, 4.953

and 6.783 respectively. The results of analysis of Aspirin, Clopidogrel, and Rosuvastatin from their tablet formulations using RP-HPLC method was found to be close to 100% in trial 14. System Suitability parameters were calculated which includes efficiency, plate count, tailing factor and % RSD. This method developed was found to be simple, accurate, specific and precise and further can be used for validation.

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