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METHOD DEVELOPMENT AND VALIDATION OF ASPIRIN AND CLOPIDOGREL PHARMACEUTICAL DOSAGE FORMS BY DEVELOPING NEW RP HPLC METHODOLOGY

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ABSTRACT
A simple and selective LC method is described for the determination of Aspirin and Clopidogrel in tablet dosage forms. Chromatographic separation was achieved on a c18 column along with mobile phase consisting of a combination of fifty five volumes of Mixed Phosphate Buffer and forty five volumes of Acetonitrile with detection of 235 nm. Linearity was observed in the range 20-60 µg/ml for Aspirin (r2=0.998) and 10-30 µg /ml for Clopidogrel (r2 =0.998) for the amount of drugs estimated by the projected ways was in smart agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD trials. All statistical data proves validity of the ways and may be used for routine analysis of pharmaceutical dosage form.

INTRODUCTION

Pharmaceutical examination implies examination of medications or synthetic elements. Webster' word reference depicts a pharmaceutical is a solution. Imaginative work (R&D) acknowledge a phenomenally thorough part in new medication switch and follow up exercises to guarantee that another prescription thing gets the made together gauges is resolute and keep embracing by definitive specialists, guaranteeing that all groups of arrangement thing are made to the particular models usage of affirmed fixings and creation technique changes into the dedication of pharmaceutical examiners in the quality control (QC) or quality affirmation (QA) division. The techniques are all around made in an investigative R&D division and exchanged to QC or assorted working environments as required. Once in a while they are exchanged to different divisions [1, 2].

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***Reverse Phase High Performance Liquid Chromatography (RP-HPLC)* [3]**

Pivot organize chromatography utilizes hydrophobic reinforced crushing, by and large with an octadecyl or octyl profitable get-together and a polar flexible stage, routinely as to some degree or absolutely fluid versatile stage. Polar substances lean toward the flexible stage and elute first. As the hydrophobic character of the solutes expands, bolster increments. By and large, the lower the farthest purpose of the versatile stage, the higher is its eluent quality. The elution request of the classes of mixes in table is rotated (subsequently the name switch organize chromatography).

Acetylsalicylic acid (ASA), (2-(acetyloxy) benzoic destructive) generally called aspirin (2-(acetyloxy) benzoic destructive), is a pharmaceutical used to treat distress, fever, and inflammation. Specific provocative conditions in which it is used fuse Kawasaki disease, pericarditis, and rheumatic fever. Migraine pharmaceutical given not long after a heart attack lessens the risk of death [4, 5]. Aspirin is moreover used whole deal to help maintain a strategic distance from heart attacks, ischaemic strokes, and blood bunches, in people at high risk. The drug may in like manner decrease the peril of particular sorts of tumor, particularly colorectal cancer. For torment or fever, impacts typically begin inside 30 minutes [6, 7].

Clopidogrel, (methyl (2S)- 2-(2-chlorophenyl)- 2-acetate) an antiplatelet administrator in a general sense and pharmacologically like ticlopidine, is used to limit blood bunches in a collection of conditions, for instance, periphery vascular disease, coronary conductor infection, and cerebrovascular infirmity. The drug is oversubscribed beneath the name medicine by Sanofi and Bristol-Myers Squibb. The prescription is an irreversible inhibitor of the P2Y12 adenosine diphosphate receptor found on the layers of platelet cells. Clopidogrel use is connected with a couple of real hostile pharmaceutical reactions, for instance, genuine neutropenia, distinctive sorts of release, and cardiovascular edema [8]. Since clopidogrel is a prodrug, it must be prepared by CYP450 chemicals to make the dynamic metabolite that controls platelet add up to. This dynamic metabolite particularly stifles adenosine diphosphate (ADP) legitimate to its platelet P2Y12 receptor and in this way the ADP-interceded activation of the glycoprotein GPIIb/IIIa complex, as needs be obstructing platelet gathering [9].

Method development is a continuous process that progresses in parallel with the evolution of the drug product. The goal and purpose of the method should reflect the phase of drug development. During early drug development, the methods may focus on API behavior. As drug development progresses, the analytical methods are refined and expanded, based on increased API and drug product knowledge. Methods should be unique and not complicated, to meet the appropriate regulatory guidelines. Method validation is the process by which it is established, through laboratory studies, that the performance characteristics of the method meet the requirements for its intended purpose. It is a part of the overall validation process that also includes software validation, instrument qualification and system suitability [10]. The main aim is to develop new RP HPLC procedure for the synchronous analysis of aspirin and clopidogrel pharmaceutical dosage outline. The plan of work includes – solubility affirmation of aspirin and clopidogrel distinctive solvents and pads, determine the digestion maxima of both the solutions in UV– Visible locale in different solvents/bolsters and picking the solvents for HPLC strategy progression. Optimize the adaptable stage and stream rates for true blue assurance and upkeep times. Validate the made technique as per ICH rules.

MATERIALS AND METHODS

Instruments Used

The instruments which were used for this work were, UV Visible apparatus which was manufactured by Nicolet evolution 100, UV-Visible Software that was developed by Vision Pro, HPLC software that was developed by Spin chrome (LC SOLUTIONS), HPLC and Electronic balance manufactured by Shimadzu(LC 20 AT VP), Ultra sonicator which was manufactured Citizen, Digital Ultrasonic Cleaner, pH meter manufactured by Global digital, Syringe was used for injection which was manufactured by Hamilton, HPLC Column was obtained from Inertsil ODS 3V(250x4.6mm) 5µm.

Reagents Used

Water, Methanol, Acetonitrile was used according to HPLC Grade, Triethyl amine, Orthophosphoric acid was used as per AR Grade.

Drugs Used

Aspirin and clopidogrel drugs were obtained as gift samples from Chandra Labs, Hyderabad. Aspirin – 75mg and clopidogrel -75mg (mg) (C-GREL-PLUS) were obtained from pharmacy.

Mobile Phase

A mix of Mixed phosphate pad (pH):ACN were prepared in the volume of 50:50 maintaining a pH of 4.0. The flexible stage was sonicated for 10 min to remove gasses and isolated through 0.45µ layer channel for degassing of compact stage.

Methodology

Determination of wavelength of aspirin and clopidogrel using UV Visible spectroscopy [11]

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic purpose is that the wavelength wherever the molar physical property is that the same for both substances that area unit interconvertible. So this wavelength is employed in synchronous estimation to estimate each medicine accurately.

Preparation of standard stock solution of aspirin

10 mg of aspirin was weighed and transferred in to 100ml volumetric flask and dissolved in water and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with water.

Preparation of standard stock solution of clopidogrel

10 mg of clopidogrel was weighed in to 100ml volumetric flask and dissolved in water and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with water.

Method Development of aspirin and clopidogrel

Trial - 1

The mobile phase that was selected for this trial is Di-Potassium phosphate K_2HPO_4 : Methanol at ratio of 55 : 45 maintaining a pH of 6.0. Weigh accurately 10 mg of aspirin and 10 mg of clopidogrel in 25 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase.[12]

From above stock solution 40µg/ml of aspirin and 20µg/ml of clopidogrel is prepared by diluting 1.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Trial- 2

The mobile phase that was selected for this trial is Di-Potassium phosphate K_2HPO_4: Acetonitrile: Methanol at ratio of 30:30:40 maintaining a pH of 4.0.weigh accurately 10 mg of aspirin and 10 mg of clopidogrel in 25 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 40µg/ml of aspirin and 20µg/ml of clopidogrel is prepared by diluting 1.5ml to 10ml with mobile phase. This solution is used for recording chromatogram. [13]

Trial- 3

The mobile phase that was selected for this trial is mixed phosphate buffer: Acetonitrile at ratio of 55: 45 maintaining a pH of 4.0.Weigh accurately 10 mg of aspirin and 10 mg of clopidogrel in 25 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase.

From above stock solution 40µg/ml of aspirin and 20µg/ml of clopidogrel is prepared by diluting 1.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.[14]

Assay

Preparation of samples for assay [15]

Preparation of mixed standard solution

Weigh accurately 10 mg of aspirin and 10 mg of clopidogrel in 25 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 40µg/ml of aspirin and 20µg/ml of clopidogrel is prepared by diluting 1.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets (each tablet contains clopidogrel-75 mg and aspirin - 75 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of clopidogrel and aspirin (µg/ml) were prepared by dissolving weight equivalent to 10 mg of clopidogrel and aspirin and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and sonicated for 5 min and dilute to 10ml with mobile phase. Further dilutions are prepared in 5 replicates of 20µg/ml of clopidogrel and 40µg/ml of aspirin was made by adding 1.5 ml of stock solution to 10 ml of mobile phase.

Validation [16]

Specificity by Direct comparison method

There is no interference of mobile part, solvent and placebo with the analyte peak and conjointly the height purity of analyte peak that indicate that the tactic is particular for the analysis of analyte's in their dosage form.

Standard sample:

Weigh accurately 10 mg of aspirin and 10 mg of clopidogrel in 25 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock

solution 40µg/ml of aspirin and 20µg/ml of clopidogrel is prepared by diluting 1.5ml to 10ml with mobile phase. This solution is used for recording chromatogram

Tablet sample

10 tablets (each tablet contains clopidogrel -75 mg and aspirin - 75 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of aspirin and clopidogrel (µg/ml) were prepared by dissolving weight equivalent to 10 mg of aspirin and clopidogrel and dissolve it in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 10ml with mobile phase. Further dilutions are prepared in 5 replicates of 20µg/ml of clopidogrel and 40µg/ml of aspirin was made by adding 1.5 ml of stock solution to 10 ml of mobile phase.

Linearity and range

Preparation of standard stock solution

Standard stock solutions of aspirin and clopidogrel (µg /ml) were prepared by dissolving 10 mg of aspirin and clopidogrel which is dissolved in sufficient mobile phase and dilute to 100 ml with mobile phase. The relationship between the concentration and area of aspirin and clopidogrel should be linear in the specified range and the correlation should not be less than 0.99.

Tablet sample

10 tablets (each tablet contains Clopidogrel-75 mg and Aspirin - 75 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of aspirin and clopidogrel (µg/ml) were prepared by dissolving weight equivalent to 10 mg of aspirin and clopidogrel and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 10ml with mobile phase. Further dilutions are prepared in 5 replicates of 20µg/ml of clopidogrel and 40µg/ml of aspirin was made by adding 1.5 ml of stock solution to 10 ml of mobile phase.

Accuracy

Accuracy of the tactic determined by recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 100%, 120%, 140%. The recovery studies were dole out thrice and also the share recovery and share mean recovery were calculated for drug is shown in table. To check the accuracy of the maneuver, recovery studies were disbursed by addition of ordinary drug resolution to pre-analyzed sample resolution at 3 totally different levels 50 %, 100%, 150%.

Precision

Prepared sample preparations of CLOPIDOGREL and ASPIRIN as per test method and injected 6 times in to the column. The % Relative standard deviation of Assay preparations of CLOPIDOGREL and ASPIRIN should be not more than 2.0%.

Limit of Detection (LOD)

The detection limit of a private analytical procedure is that the lowest quantity of analyte which may be detected however not essentially quantitated as a precise worth. It is a limit check that specifies whether or not associate analyte is on top of or below an exact worth. The standard deviation of the response can be determined based on the standard deviation of the blank, on the residual standard deviation of the regression line, or the standard deviation of y-intercepts of regression lines.

Limit of Quantitation (LOQ)

The limit of quantitation is outlined because the lowest concentration of associate analyte which will be determined with acceptable preciseness and accuracy underneath the explicit operational conditions of the method. Again, the standard deviation of the response can be determined based on the standard deviation of the blank, on the residual standard deviation of the regression line, or the standard deviation of y-intercepts of regression lines.

Robustness

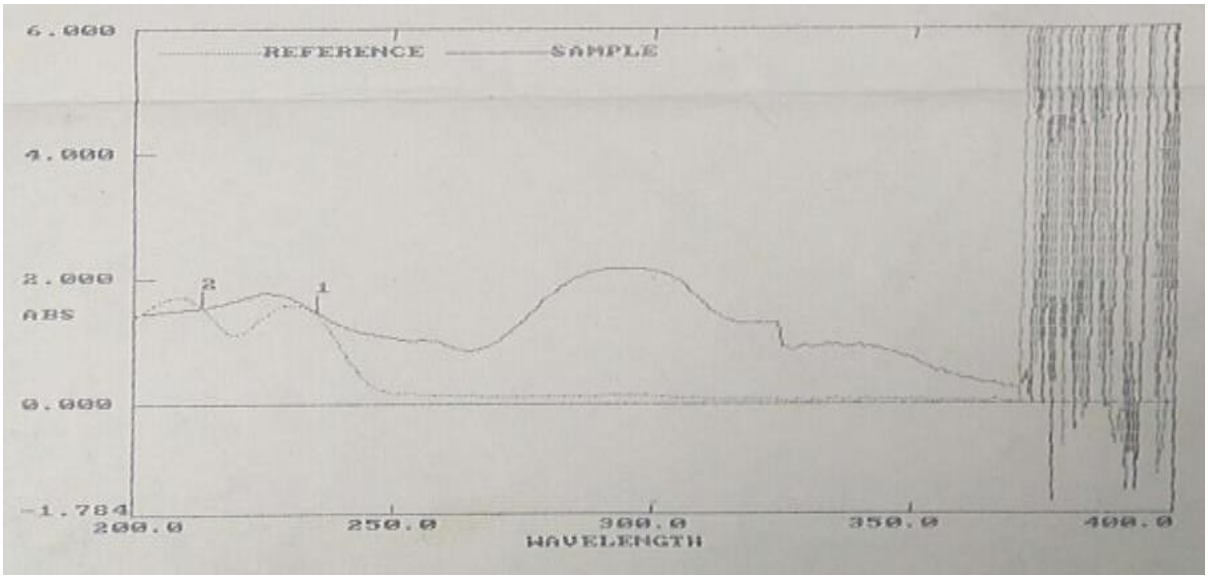
To demonstrate the strength of the tactic, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and wavelength. System suitableness parameters were compared therewith of technique preciseness. The system suitableness ought to pass as per the check technique at variable conditions.

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the assay by two different analysts. The % Relative standard deviation of assay values between two analysts should be not more than 2.0%.

RESULTS AND DISCUSSIONS

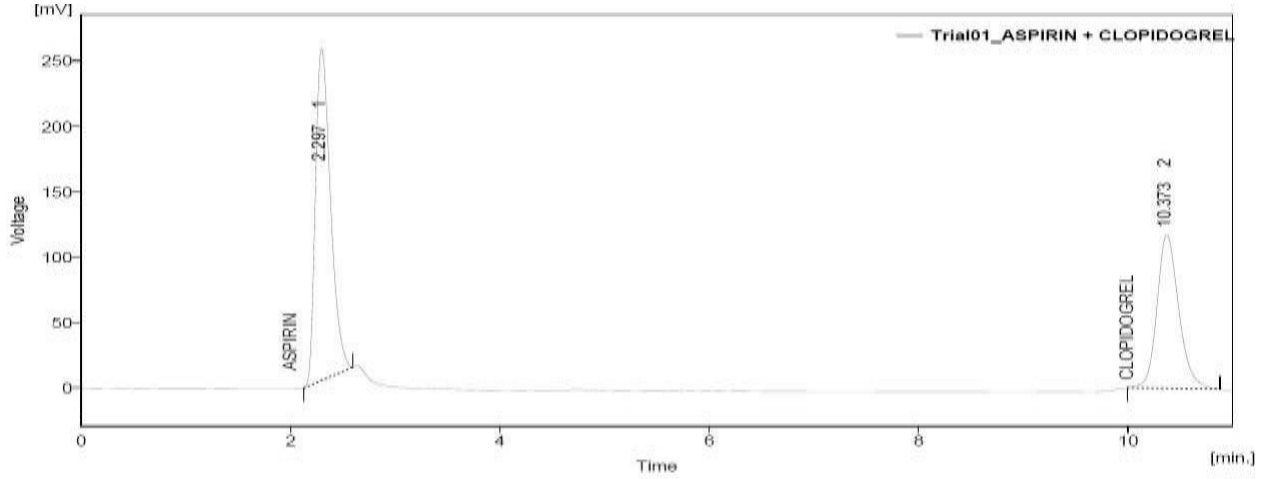
Determination of wavelength of aspirin and clopidogrel using UV Visible spectroscopy



(X-axis – Wavelength and Y – axis – absorbance)

The Isobestic point was found to be 235 nm for aspirin and clopidogrel in combination

Method Development of aspirin and clopidogrel – Trial – 01. (X – Axis – Time, Y – axis – Voltage)



Result Table (Uncal - Trial01_ASPIRIN + CLOPIDOGREL

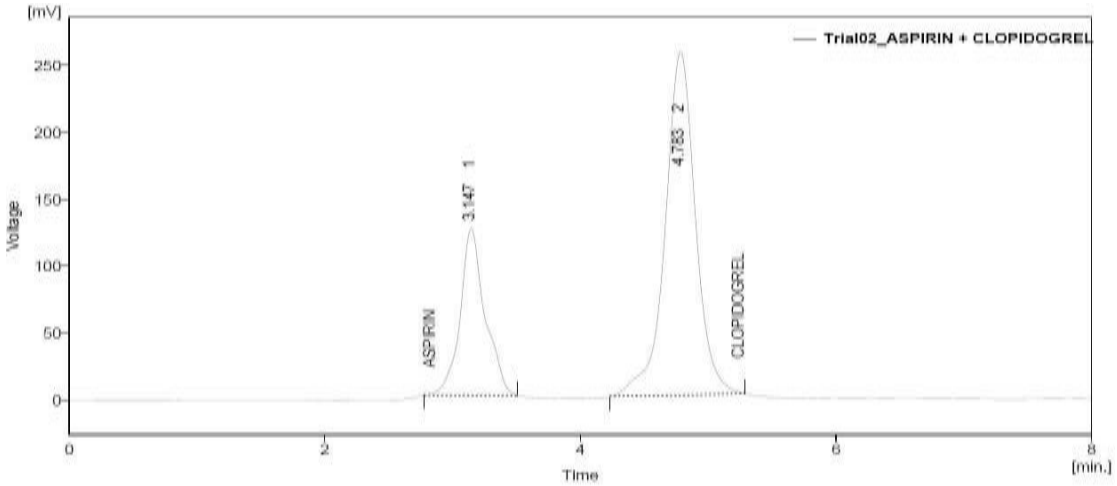
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.297	2546.935	253.669	60.0	68.3	0.16
2	10.373	1699.517	117.670	40.0	31.7	0.22
	Total	4246.452	371.340	100.0	100.0	

Column Performance Table (From 50% - Trial01_ASPIRIN + CLOPIDOGREL

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.297	0.160	1.781	0.00	1141	11415	-
2	10.373	0.220	1.327	0.00	12317	123169	25.013

Although the Efficiency was not satisfactory for aspirin and the peak response of clopidogrel was very less. The retention time is more. Hence it was not taken for optimization.

Method Development of aspirin and clopidogrel – Trial – 02. (X – Axis – Time, Y – axis – Voltage)



Result Table (Uncal - Trial02_ASPIRIN + CLOPIDOGREL

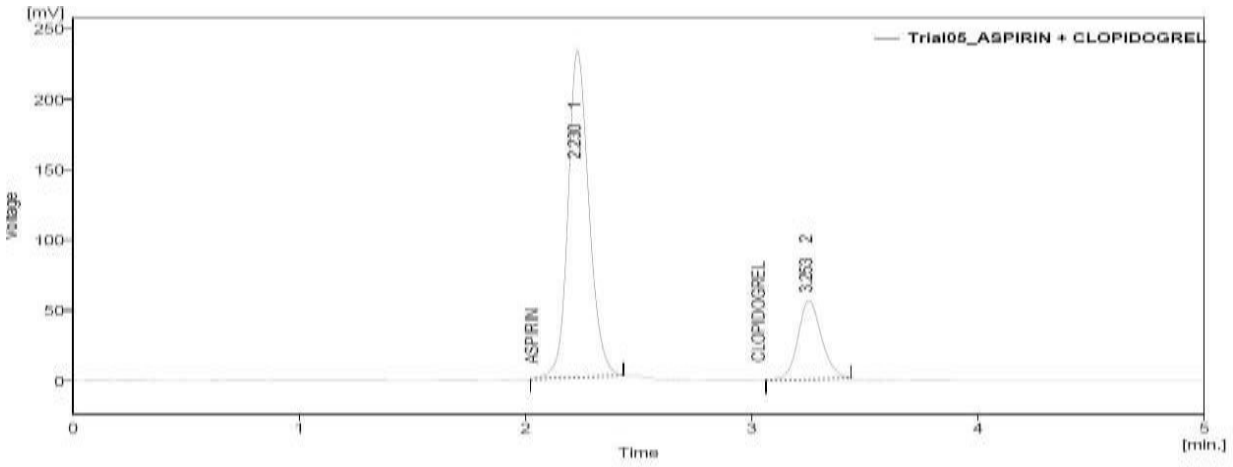
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	3.147	1687.607	124.641	28.5	32.7	0.18
2	4.783	4242.123	256.761	71.5	67.3	0.24
	Total	5929.730	381.402	100.0	100.0	

Column Performance Table (From 50% - Trial02_ASPIRIN + CLOPIDOGREL

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	3.147	0.183	1.271	0.00	1632	16320	-
2	4.783	0.243	1.014	0.00	2141	21408	4.514

The efficiency of aspirin was not satisfactory. Hence it was not taken for optimization.

Method Development of aspirin and clopidogrel – Trial – 03. (X – Axis – Time, Y – axis – Voltage)



Result Table (Uncal - Trial05_ASPIRIN + CLOPIDOGREL

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.230	1490.503	231.652	78.6	80.5	0.10
2	3.253	407.001	56.002	21.4	19.5	0.11
	Total	1897.504	287.655	100.0	100.0	

Column Performance Table (From 50% - Trial05_ASPIRIN + CLOPIDOGREL

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.230	0.097	1.320	0.00	2948	29483	-
2	3.253	0.113	1.276	0.00	4565	45651	5.736

All the system suitability requirements were met. The peak Asymmetry factor was less than 2 for both aspirin and clopidogrel. The efficiency was more than 2000 aspirin and clopidogrel .Resolution between two peaks >1.5. Hence this method was for optimized.

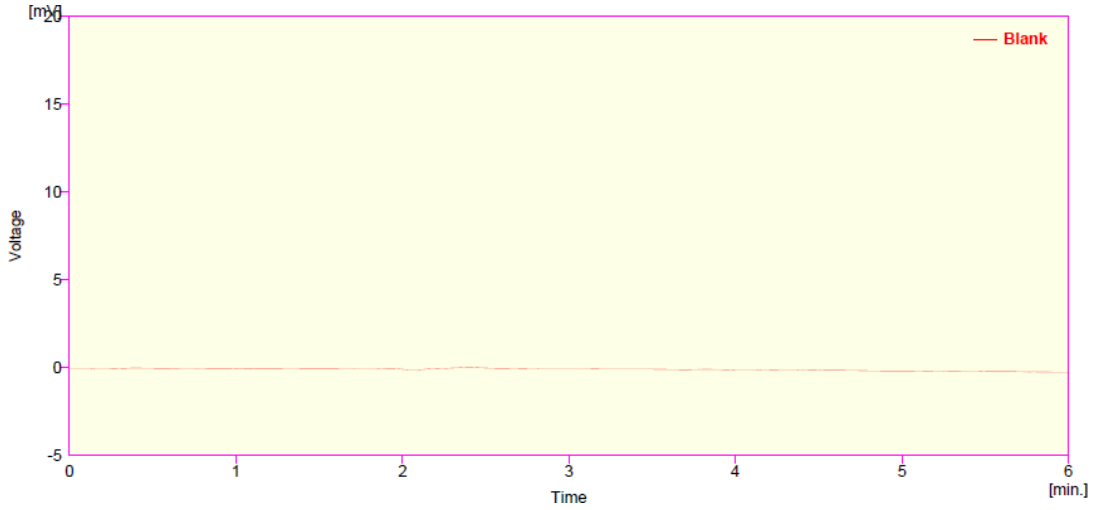
Assay Results

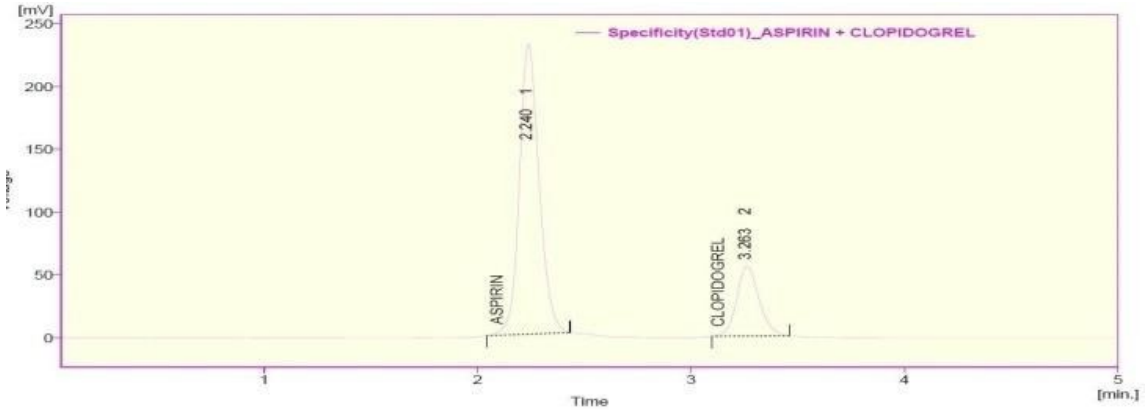
	Aspirin		**Clopidogrel**	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	1487.1	1487.447	410.632	409.505
Injection-2	1483.265	1488.48	404.609	409.228
Injection-3	1488.429	1483.804	409.31	408.099
Injection-4	1489.131	1488.429	411.211	409.31
Injection-5	1483.538	1487.1	407.001	405.125
Average Area	1486.293	1487.052	408.5526	408.2534
Assay(%purity)	100.051094		99.9267659	

The amount of aspirin and clopidogrel present in the taken dosage form was found to be 100.05 % and 99.92 % respectively.

Validation

Specificity by Direct comparison method (X – Axis – Time, Y – axis – Voltage) (Blank and Sample)



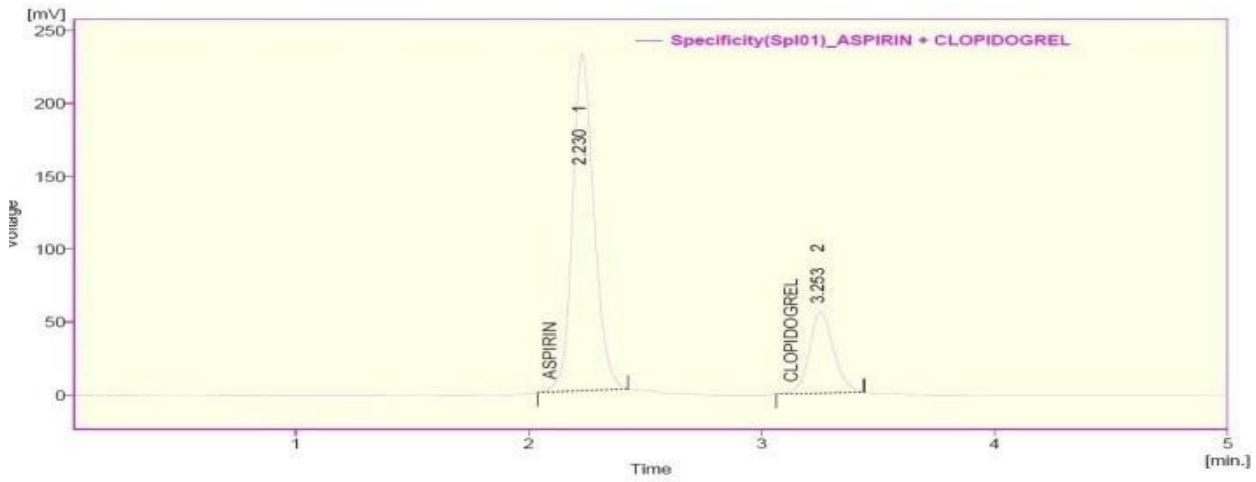


Result Table (Uncal - Specificity(Std01)_ASPIRIN + CLOPIDOGREL

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.240	1487.970	230.909	78.51
2	3.263	407.236	55.717	21.49
	Total	1895.206	286.626	100.00

Column Performance Table (From 50% - Specificity(Std01)_ASPIRIN + CLOPIDOGREL

	Reten. Time	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.240	0.100	1.231	2780	27798	-
2	3.263	0.113	1.276	4593	45932	5.645



Result Table (Uncal - Specificity(Spl01)_ASPIRIN + CLOPIDOGREL

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.230	1483.538	231.359	78.47
2	3.253	407.001	56.002	21.53
	Total	1890.538	287.362	100.00

Column Performance Table (From 50% - Specificity(Spl01)_ASPIRIN + CLOPIDOGREL

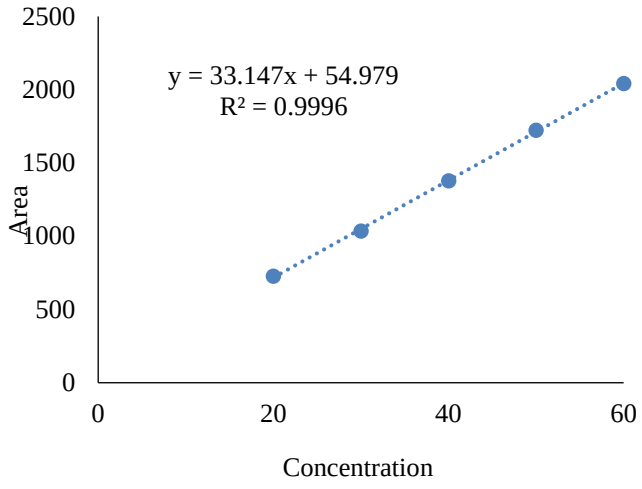
	Reten. Time	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.230	0.097	1.320	2948	29483	-
2	3.253	0.113	1.276	4565	45651	5.735

It is observed from the above data, diluent or excipient peaks are not interfering with the aspirin and clopidogrel peaks.

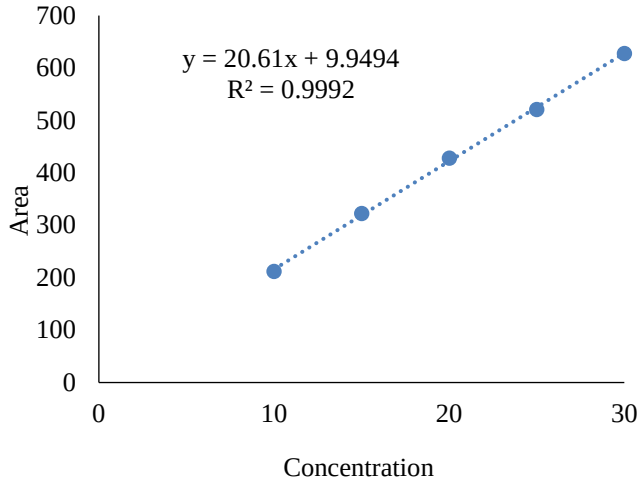
Linearity of Aspirin and Clopidogrel

	Aspirin		Clopidogrel	
S. No.	**Conc.(µg/ml)**	**Area**	**Conc.(µg/ml)**	**Area**
1	20	727.341	10	211.784
2	30	1035.752	15	322.204
3	40	1377.14	20	428.317
4	50	1722.958	25	520.564
5	60	2041.082	30	627.846

Aspirin



Clopidogrel



The correlation coefficient for linear curve obtained between concentrations vs. Area for standard preparations of aspirin and clopidogrel is 0.998 and 0.998. The relationship between the concentration of aspirin and clopidogrel and area of aspirin and clopidogrel is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.

Accuracy results of aspirin

Recovery level	Accuracy Aspirin			Average % Recovery
	Amount taken(mcg/ml)	Area	%Recovery	
50	20	410.811	197.6411668	
	20	411.371	197.9105828	102.83
	20	411.224	197.8398611	
100	40	522.084	125.5873028	
	40	527.341	126.8518741	99.36
	40	529.651	127.4075446	
150	60	621.351	89.67958741	
	60	621.351	89.67958741	99.103
	60	615.264	88.80105072	

The percentage mean recovery of aspirin and clopidogrel is 99.19 and 99.89 % respectively.

Results for Method Precision of aspirin and clopidogrel

S. No.	Aspirin		Clopidogrel	
	Rt	Area	Rt	Area
1	3.145	978370.000	6.211	340457
2	3.165	962064.000	6.224	341907
3	3.151	967422.000	6.212	339323.000
4	3.148	955774.000	6.194	339473.000
5	3.126	951906.000	6.168	339074
6	3.116	962532.000	6.170	340503.000
Avg	3.1418	963011.333	6.197	340122.833
St dev	0.0178	9297.067	0.023	1058.443
%RSD	**0.57**	**0.97**	**0.38**	**0.31**

Test results for Clopidogrel and Aspirin are showing that the %RSD of Assay results are within limits.

Result of Robustness study

Parameter	Aspirin		Clopidogrel	
	Retention time(min)	Tailing factor	Retention time(min)	Tailing factor
Flow Rate				
0.8 ml/min	2.562	1.679	5.059	1.263
1.2 ml/min	2.148	1.678	4.235	1.264
Wavelength				
233nm	2.566	1.687	5.052	1.262
237nm	2.570	1.686	5.065	1.265

From the observation it was found that the system suitability parameters were within limit at all variable conditions.

Results for Ruggedness

Aspirin	**%Assay**	**Clopidogrel**	**%Assay**
Analyst 01	100.5	**Analyst 01**	98.9
Anaylst 02	99.5	**Anaylst 02**	100.6

From the observation the between 2 analysts Assay values not higher than 2.0%, therefore the strategy was rugged.

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation aspirin and clopidogrel was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

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