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DEVELOPMENT AND VALIDATION OF A RP HPLC METHOD FOR ESTIMATION OF CLOPIDOGREL AND ASPIRIN IN BULK AND IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

The purpose of these work was to develop a simple, rapid, sensitive and validated RP-HPLC method for separation and analysis of Aspirin and Clopidogrel in bulk and tablet dosage form. The separation was conducted by using Grace C 18 (150×4.6µm) with mobile phase consisting of methanol: water in the ratio (90:10) pH4. The mobile phase was delivered at the flow rate of 1.0 ml/min. The eluent was monitored at wavelength 244 nm by UV 3000 wavelength detector and found a sharp and symmetrical peak with retention time for Aspirin and Clopidogrel was found to be 4.474 min and 5.883 min respectively. The method was validated for linearity, accuracy, precision, system suitability, and stability. These method was validate according to ICH guidelines which include linearity, precision, accuracy, robustness, LOD, LOQ. The result obtained were within the acceptance criteria as per ICH guidelines.

KEYWORDS: HPLC, Aspirin, Clopidogrel, Methanol, Water, Validation.

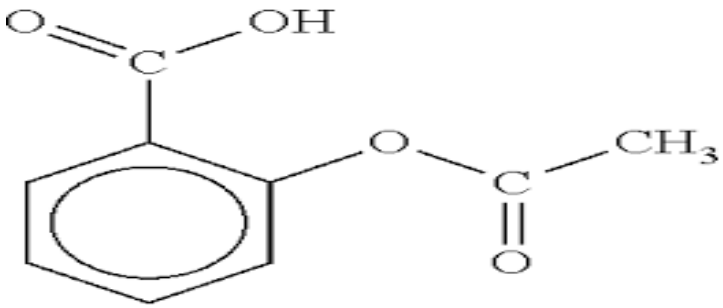
INTRODUCTION

The antiplatelet therapy for prevention of thrombotic events in cardiovascular disease are evident. Statistical studies have shown that secondary prevention by antiplatelet agent reduces risk of myocardial infraction and stroke.[1]

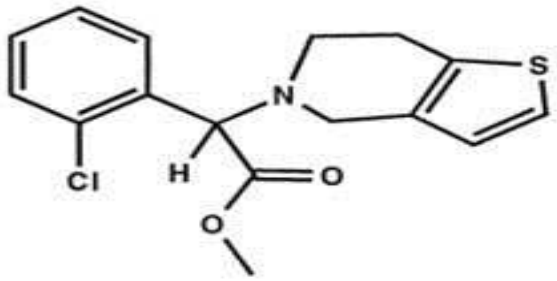
Clopidogrel is used to reduce the risk of heart disease and sroke in those at high risk. it is also together with aspirin in heart attacks and following the placement of a coronary artery.[2] Aspirin is the most widely used antiplatelet agent which inhibites platelet cyclo-oxygenase and the conversion of arachidonic acid to the potent platelet agonist thromboxane A but not prevent platelet activation occurring via various signaling pathways that are independent of thromboxane A release. Therefore a number of other compounds have been developed to complement aspirins beneficial effect. these includes thiopyridines like clopidogrel.[3]

Aspirin irreversibly inhibits cyclo-oxygenase in platelet and therefore blocks the formation of thromboxane A and the clopidogrel is metabolized in liver to its active compound which covalently bind to the adenosine phosphate receptor on platelet and dramatically reduces platelet aggregation. Clopidogrel is a prodrug, which is activated in two steps, first by CYP2C19, CYP1A2 AND CYP2B6, then by CYP2C19, CYP2C9, CYP2B6 and CYP3A steps. The active metabolite then specifically and irreversibly inhibits the P2Y12 subtype of ADP receptor, which is important in activation of platelet and eventual cross linking by the protein fibrin.[2]

Structure of Aspirin



Structure of Copidogrel



Literature survey revealed several assay methods for ASP and CLO individually or in combination with other drugs by, RP HPLC-UV[4,5,6,7,8], Ion pairing RP-HPLC[9], HPTLC[10,11], Gas Chromatography-Mass Spectrometry (GC-MS) LC-MS/MS.[12] Reverse phase -Ultra fast liquid chromatography (RP-UFLC)[13], UV spectrophotometry.[14] However every method has its own limitation. Thereby, an attempt has been taken to develop and validate a simple, economic, reliable, reproducible, accurate and precise RPHPLC based method for simultaneous estimation of ASP and CLO in combined tablet dosage form where validation of the analytical method has been performed in accordance with ICH guideline.[19]

MATERIAL AND METHOD

Table no. 1: Drug name.

Drug (1)	Aspirin
Drug (2)	Clopidogrel

Table No. 2: Reagent and chemicals.

Sr. no.	Name of reagent used	Make
1.	Water	Analytical and HPLC grade
2.	Methanol	Analytical and HPLC grade
3.	Acetonitrile	Analytical and HPLC grade

Table No. 3: List of instruments.

Sr. no.	Name of Equipment	Source
1.	HPLC	Analytical technology Ltd. HPLC3000 system
2.	UV	Jasco V-630
3.	Detector	UV 3000 M
4.	Balance	Wensor high precision balance
5.	Sonicator	Wensor ultra sonicator
6.	pH meter	Thermo orion 4 star

VALIDATION OF DEVELOPED METHOD

1. SYSTEM SUITABILIY TEST

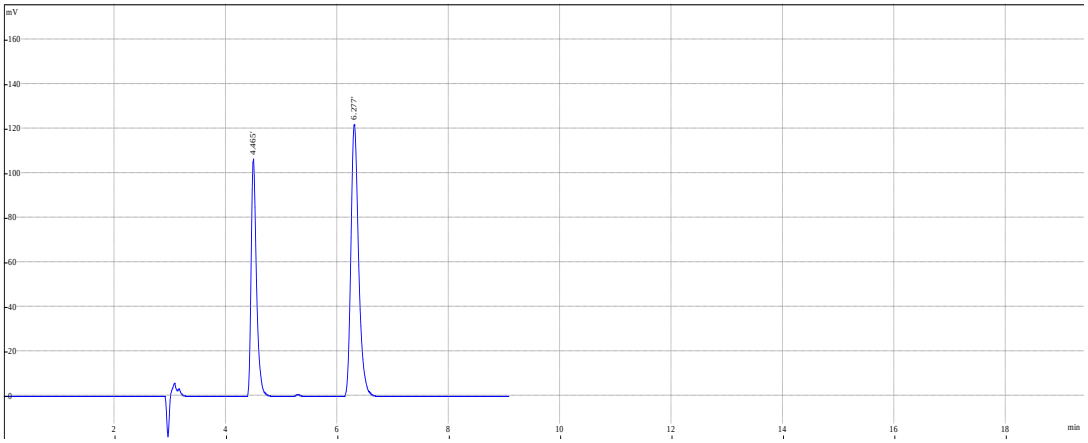
HPLC system was optimized as per chromatographic condition. 20µ/ml of working standard solution of drugs were injected triplicate into the chromatographic system. The chromatogram were recorded and majored the response for major peak. system suitability parameter such as retention time, Theoretical plate and Asymmetry factor. then the %RSD of all parameter were calculated.

Table no 4: Data for system suitability for Aspirin and Clopidogrel.

system suitability parameters	Aspirin	Clopidogrel
Retention time	4.474	5.883
therotical plate	7769	8592
Asymentry factor	1.26	1.24
Resolution	4.58	4.58

2. ASSAY (%)

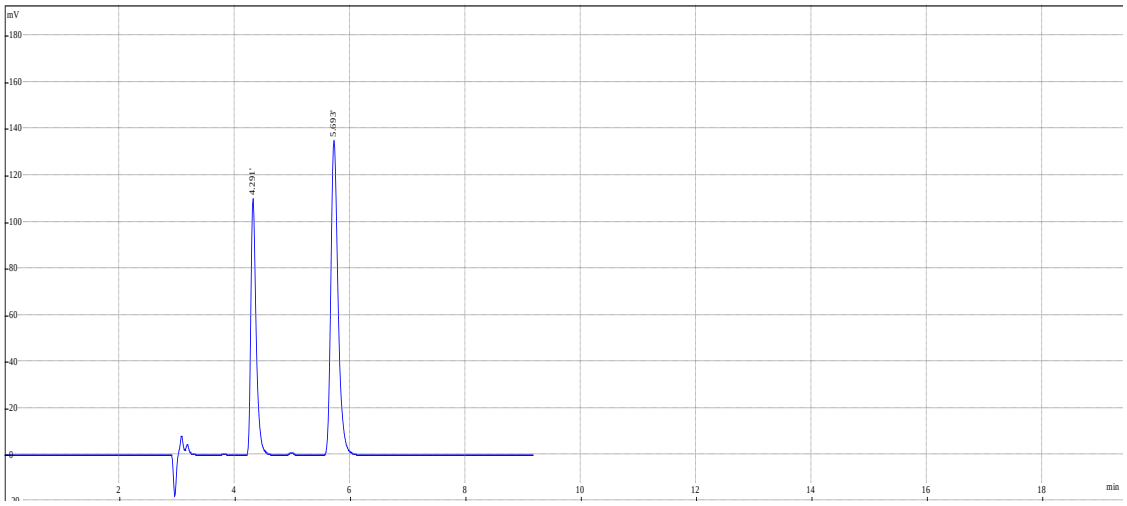
A. Standard drug of Aspirin and Clopidogrel



Time	Conc.	Area	Resolution	T. Plate	Asymmetry
4.465	30	1154451	5.47	8644	1.24
6.277	30	1488773	0.00	7957	1.22

Fig. No. 1: Chromatogram for standard drug of aspirin and clopidogrel.

B) Sample of Aspirin and Clopidogrel



Time	Conc.	Area	Resolution	T. Plate	Asymmetry
4.291	30	1152870	5.03	8279	1.26
5.693	30	1487912	0.00	8301	1.19

Fig. No. 2: Chromatogram for sample of aspirin and clopidogrel.

Table 22: Assay of Aspirin.

Sr. No.	Conc.	Area of standard	Area of sample	% Assay
1	30	1154451	1152870	99.86

Table 23: Assay of Clopidogrel.

Sr. No.	Conc	Area of standard	Area of sample	% Assay
	30	1488773	1487912	99.94

OBSERVATION

The individual assay of Aspirin and Clopidogrel was found within a limit, should not less than 98% and not more than 102%.

3. LINEARITY

Linearity was tested for range of concentration 10 to 50 µ/ml for Aspirin and Clopidogrel respectively Each sample was analysed and peak areas were recorded. The response factor was plotted against the concentration of Aspirin and Clopidogrel obtain in calibration curve for Aspirin and Clopidogrel respectively.

Table no. 5: linearity range study results for aspirin and clopidogrel.

Parameter	Aspirin	Clopidogrel
Linearity range	10 to 50 µ/ml	10 to 50 µ/ml
Correlation coefficient	0.9988	0.9988

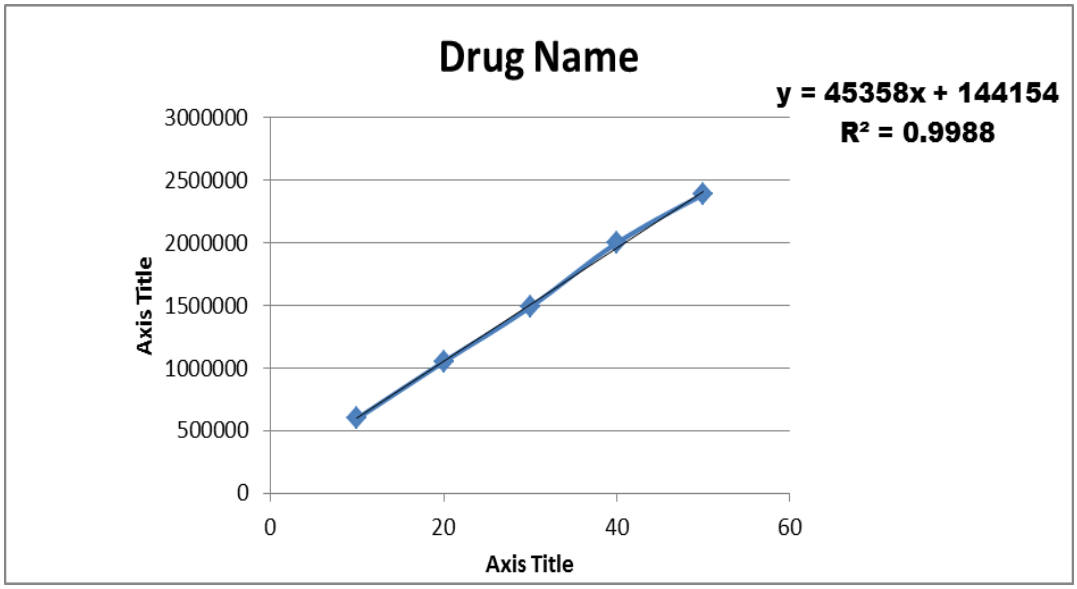


Fig. no. 3: Linearity graph of Aspirin.

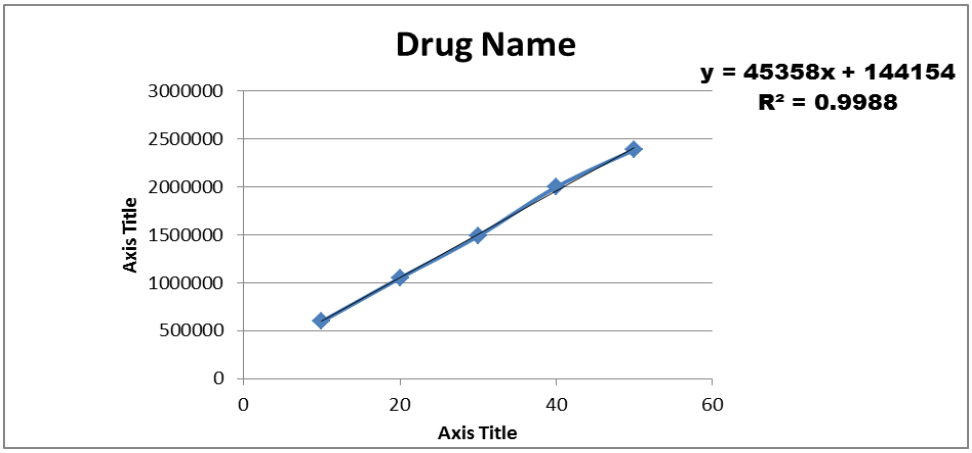


Fig no. 4: Linearity graph of Clopidogrel.

4. PRECISION

The precision of an analytical method is the degree of agreement among individual test result when the procedure is applied repeatedly to multiple sampling of a homogenous sample under prescribed condition. Precision of the method was determined by Repeatability (intraday) and intermediate precision (interday) studies .the low RSD value indicate that the method was précised. the relative standard deviation should not more than **2.0%.**

Table no. 6: Inter day variability for Aspirin and Clopidogrel.

Aspirin		Clopidogrel
Day1	**Area**	**Area**
	1151439	1485759
	1156192	1483034
	1150656	1483943
Day 2	1151439	1485759
	1156192	1483034
	1150656	1483943
Mean	11506556	1483943
%RSD	0.23%	**0.08%**

Table no. 7: Intraday variability for Aspirin and Clopidogrel.

Aspirin		Clopidogrel
Morning	**Area**	**Area**
	1154506	1491316
	1149374	1491217
	1155726	1493350
Evening	1154506	1491316
	1149374	1491217
	1155726	1493350
Mean	1153202	1491961
%RSD	0.26%	0.09

5. ACCURACY: (% RECOVERY)

The accuracy of the method was determined by calculating the recoveries of Aspirin and Clopidogrel by standard addition method at the three concentration level the percentage recovery of Aspirin and Clopidogrel were fount to be in a range. The result of accuracy studies for Aspirin shown in table no. 8 and Clopiogrel shown in table no. 9.

Table no. 8: Recovery of Aspirin.

Composition	Sample amount (ppm)	Amount added (ppm)	Amount recovered (ppm)	% Recovery	%mean recovery
50%	20	10	29.93	99.76	100.1
	20	10	30.33	101.1	
	20	10	29.96	99.86	
100%	20	20	39.89	99.72	99.84
	20	20	39.96	99.9	
	20	20	39.96	99.9	
150%	20	30	50.07	100.14	100.06
	20	30	50.05	100.1	
	20	30	49.97	99.94	

Table no. 9: Recovery of Clopidogrel.

Composition	Sample amount (ppm)	Amount added (ppm)	Amount recovered (ppm)	%Recovery	%mean recovery
50%	20	10	30.04	100.1	99.89
	20	10	30.01	100.03	
	20	10	29.87	99.56	
100%	20	20	40.16	100.4	100.10
	20	20	40.16	100.4	
	20	20	39.81	99.52	
150%	20	30	50.06	100.12	99.86
	20	30	49.84	99.68	
	20	30	49.89	99.78	

6. ROBUSTNESS

Robustness of the method were determined by changing the method parameter (wavelength and pH).the result were presented in following table based on the result of these studies shows, the small change made to the method procedure ,but it was not affect the method result so this method is robust.

Table no. 10: Data of different wavelength.

Drug sample	wavelength	Area	Mean	SD	%RSD
ASPIRIN	244nm	1064719	896782.7	145458.9	0.80
	242nm	815331			
	246nm	810298			
CLOPIDOGREL	244nm	1601923	1233764	318843	0.99
	242nm	1047390			
	246nm	1051980			

Table no. 11: Data of different PH.

Drug sample	PH	Area	Mean	SD	%RSD
ASPIRIN	4.0	1064719	897860.3	144506.1	0.78
	3.8	815230			
	4.2	813632			
CLOPIDOGREL	4.0	1601923	1235123	317662.2	0.83
	3.8	1049982			
	4.2	1053464			

7. RUGGEDNESS

Acceptance criteria for the ruggedness is not more than 2%.

Table no. 12: Ruggedness data of Aspirin.

Sr.no	Concentration	Area
1	10	450700
2	20	816702
3	30	1159481
4	40	1522909
5	50	1841098

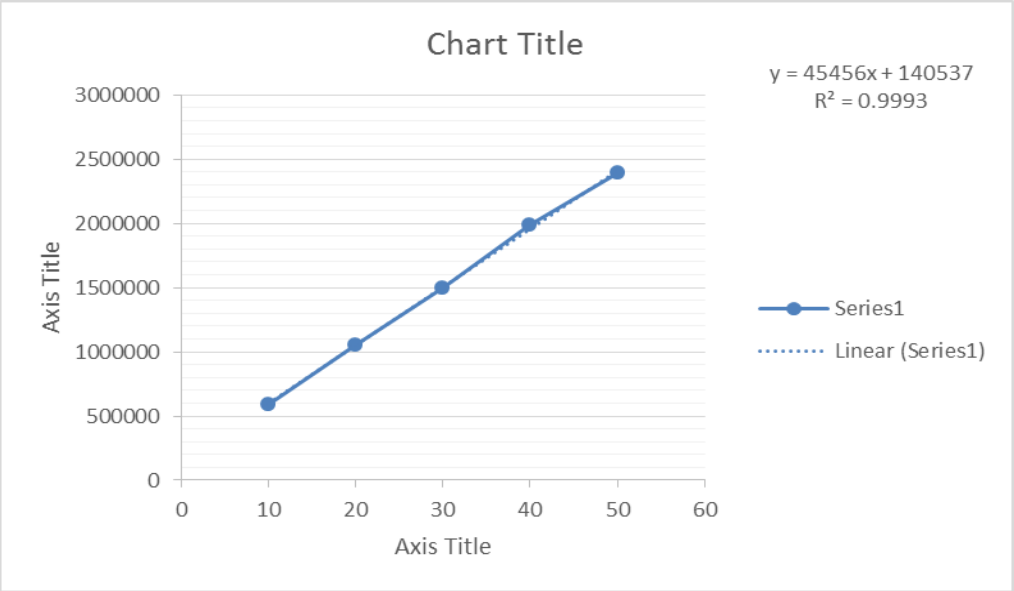


Fig no. 5- Aspirin.

Table no. 13: Ruggedness data of Clopidogrel.

Sr.no	Concentration	Area
1	10	590971
2	20	1052298
3	30	1492815
4	40	1990222
5	50	2394824

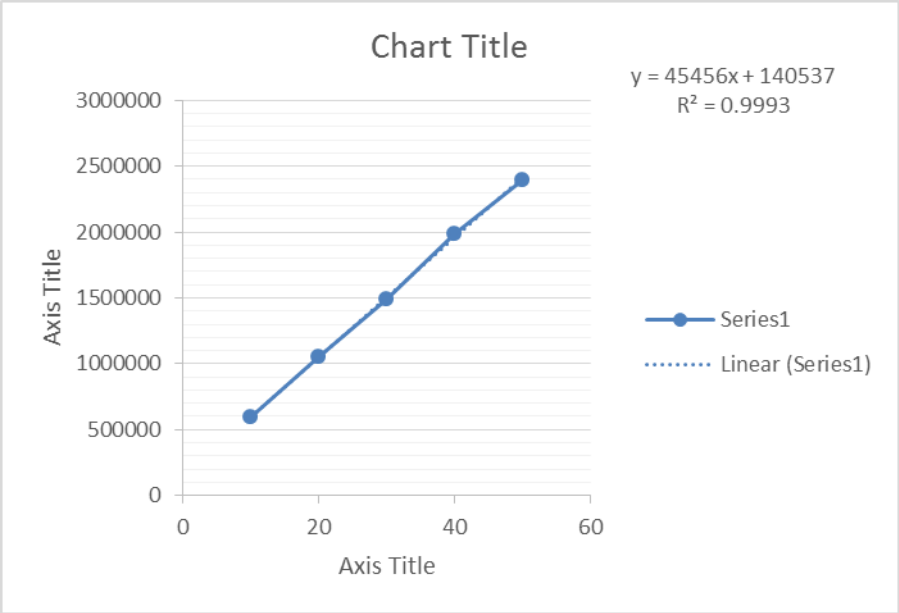


Fig no. 6: Clopidogrel.

8. LIMIT OF DETECTION AND LIMIT OF QUANTITATION (LOD and LOQ)

From the linearity data calculate the limit of detection and limit of quantification,

Table no 14: LOD and LOQ.

Parameters	Aspirin	Clopidogrel
LOD	0.0662	0.1029
LOQ	0.200	3.059

SUMMARY AND CONCLUSION

The proposed HPLC method was found to be simple, linear, précised, accurate and robust for the simultaneous estimation of Aspirin and Clopidogrel in bulk and in pharmaceutical dosage form. Grace C18 column was used as stationary phase. Mobile phase consisting of mixing Methanol and Water (90:10V/V) pH-4. Retention time of Aspirin and Clopidogrel was found to be 4.474 min and 5.883 min respectively. The developed method was validated based on ICH guidelines, the linearity of Aspirin and Clopidogrel was found to be in concentration range 10 to 50 µg/ml respectively. Precision and robustness of developed method, %RSD

was found to be less than 2%. The LOD of Aspirin and Clopidogrel was 0.0662 µg/ml and 0.1029 µg/ml and LOQ were 0.200µg/ml and 0.359 µg/ml. The % recovery was found to be 2892.4% and 44.97% respectively. % Assay of Aspirin and Clopidogrel was found to be 99.86% and 99.94% respectively.

The developed RP-HPLC method was found to be simple, précised, linear, accurate and robust according to the acceptance criteria. The method was found to be better than previously reported method because of its UV detection and better resolution of peak. The coefficient correlation was found in acceptance range. Obtained results are good agreement with declared contents of dosage formulation, result are accurate and précised and are confirmed by statistical parameter. thus from the above result of the individual test is conclude that the analytical method is validated and found to be satiafactory.

REFERENCES

1. Enma V. Paez Espinosa, John P.Murad, and Fadi T. Khasawneh, Aspirin: Pharmacology and Clinical Applications, HPCT, 2012; 1-15.
2. http://enwikipedia.org/wiki/clopidogrel.
3. Graeme J Hankey and John W Eikelboom, antiplatelate drugs, Med J Australia, 2003; 178(11): 568-574 Published online: 2 June 2003.
4. Shrivastava PK, Basniwal PK, Jain Deepti and Shrivastava SK, Concurrent Estimation of Clopidogrel Bisulfate and Aspirin in Tablets by Validated RP-HPLC Method; IJPS, 2008; 1-5.
5. Masud Al Abdullah and Iris Begum, Development and validation of a RP-HPLC method for simultaneous estimation of aspirin and clopidogrel in combined tablet dasage form; IJPSR, 2016; 7(11): 4443-4448.
6. Mounika A, Shriram N, Method development and validation of clopidogrel bisalphate By RP-HPLC in bulk; IJPAR, 2012; 1(1): 1-7.
7. Anandakumar K, Ayyappan T, Raghu raman V, vetrichelvan T, Sankar ASK, and Nagavalli D, RP-HPLC Analysis of aspirin and clopidogrel bisulphate in combination; Indian J pharm Sci, 2007; 69(4): 597-599.
8. Najma Sultana, Muhammad S. Arayne, Kiran A., Ali, and Muhammad Nawaz, Simultaneous Determination of Clopidogrel and Aspirin by RP-HPLC from Bulk Material and Dosage Formulations Using Multivariate Calibration Technique; Journal of Chromatographic Science, 49, February 2011.

9. Sagar S.Panda, Ion-Pairing RP-HPLC Method for Simultaneous determination of Aspirin and Clopidogrel bisulphate in Tablet and Capsule Dosage Form; International Journal of Pharm Tech Research CODEN (USA): IJPRIF ISSN: 0974-430 Jan-Mar 2010; 2(1): 269-273.
10. Purushotam K. Sinha, Mrinalini C. Damle 1 and K.G. Bothara, A Validated Stability Indicating HPTLC Method for Determination of Aspirin and Clopidogrel Bisulphate in Combined Dosage Form; Eurasian J. Anal. Chem, 2009; 4(2): 152-160.
11. Mrinalini C.Damle, Amol O.Bajaj, Asma Y.Saudagar, Nikhil T.Awatade, Ruchira K.Makawana, Padmanabh B.Deshpande, A validated high performance thin layer chromatographic method for simultaneous estimation of aspirin and clopidogrel in pharmaceutical dosage form; ACAIJ, 2008; 7(11): 789-794.
12. Pravinkumar P., Shrikant I., Anushka k., Shrivasa rao D., LC, method development and validation of aspirin and clopidogrel in pure APIs and its pharmaceutical dosage form: IJPAR, Dec. 2014; 3(4): 2320-2831.
13. Jinesh Bahubali Nagavi, Bannimath Gurupadayya, Preethi G., validated bio-analytical method development for simultaneous estimation of clopidogrel and aspirin in human plasma by rp-ultra fast liquid chromatography: world journal of pharmacy and pharmaceutical sciences SJIF, 2014; 3(9): 518-531.
14. Sunil Singh, Nitin Dubey and D.K. Jain, Simultaneous Estimation of Atorvastatin, Clopidogrel and Aspirin in Capsule Dosage forms using UV-Spectroscopy: Asian J. Research Chem, Oct. - Dec. 2010; 3(4): 885-888.
15. https://en.Wikipedia.org/Wiki/Aspirin
16. Behan MWH, storey RF, Antiplatelet therapy in cardiovascular disease; spostgraduate Medical journal, 2004; 80: 155-164.
17. https://pubmed.ncbi.nlm.nih.gov/compound/clopidogrel#section=Top.
18. Tripathi KD. Essentials of medical pharmacology. 5th ed. Jaypee Brothers Medical Publishers (P) Ltd New Delhi, 2004; 560.
19. Proceedings of the International Conference on Harmonization. Geneva: 1996. Mar, ICH, Q2B, Harmonized Tripartite Guideline, Validation of Analytical Procedure: Methodology, IFPMA.
20. Raymond PW Scott; Principles and Practice of Chromatography, 2003; 1-4.
21. Ewing GW, Instrumental methods of chemical analysis, 5th ed, Mcgraw Hill International Edition 1985, Chemistry Series, 1-4.

22. Connor KA, A textbook of pharmaceutical analysis, 3rd ed, John Wiley and Sons Publication, 1999; 373; 173-179.
23. Skoog DA, West DM and Holler FT, Fundamentals of analytical chemistry, 7th ed, Harcourt College Publication, 2001; 710.
24. Skoog DA, West DM and Holler F.T, Fundamentals of analytical chemistry, 8th ed, Harcourt College Publication, 2001; 2,710, 836, 1973.
25. Kasture AV Wadodkar SG, Mahadik KR, More HM, Pharmaceutical Analysis, 2nd ed, 1997; (1): P. 1, 4, 48, 169.
26. Bekett AH, Stenlake JB, Practicle Pharmaceutical chemistry, 4th ed, CBS Publishers New Delhi 2004, Part-II, P. 275-314.
27. Sharma YR, Elementary organic spectroscopy principal and chemical analysis, S. Chand publication, 2002; 9.
28. Kemp W, Organic Spectroscopy, 2nd ed, ELBS publication, 1989; 194.
29. Sharma BK, Instrumental Methods of Chemical Analysis, Introduction to Analytical Chemistry, Goel publishing house, Meerat, 19th edition, 2000; 1-4: 200-203.
30. Chatwal GR, Sham KA, Instrumental method of chemical analysis, 5th ed, Himalaya publication, P. 2.149, 2.566, 2.624.
31. Nash RA and Wachter AH, Pharmaceutical Process Validation, An International 3rd ed, 129.
32. CDER. Reviewer Guidance. "Validation of Chromatographic Methods." 1994 November.
33. Ahuja S, Stephen Scypinski, Handbook of Modern Pharmaceutical Analysis, 86-167, 445-482.
34. http;//www.drugbank.drugs/DB0067
35. 28. http://en.wikipedia.org/wiki/Aspirin
36. http://en.wikipedia.org/wiki/ clopidogrel