Int. J. Pharm. Investigation., 2023; 13(2):270-278.
https://www.jpionline.org

Original Article

Stability Indicating HPLC Method for Simultaneous Assessment of Clopidogrel Bisulfate and Aspirin: Development and Validation

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ABSTRACT

Objectives: The efforts were made to develop a HPLC analytical method for the simultaneous quantitative estimation of aspirin and clopidogrel and aim to identify and estimate the degradation of the drugs under the various stress conditions recommended by ICH guideline. **Materials and Methods:** The separation of two drugs were done by using LC-20AD liquid chromatograph having SPD-20A UV-vis detector on C_{18} (4.6 x 150 mm) column which was connected with loop 20µl and with HPLC-Dell system. Mobile phase consisted of acetonitrile: buffer in the ratio of 350:650 v/v. Flow rate was 1.3 ml/min and detection were done at 220 nm. Proportions of solvents and adjustment of mobile phase was carried out by screening. **Results:** The chromatographic separation of aspirin was noted at 4.299 minutes with average USP tangent of 8332.046 and clopidogrel was at 12.706 min with average of 11886.0397 tangent. A linear correlation was observed between concentration of aspirin and clopidogrel with their dilutions i.e r^2 = 0.9986 and 0.9996 respectively. The outcome of study, with limit of detection 0.058 and 0.078 µg/ml and limit of quantification 0.117 and 0.156 µg/ml, for aspirin and clopidogrel revealed the repeatability, reproducibility and robustness of the method. Stability indicating parameters were tested by ICH guideline. **Conclusion:** It is concluded that the method is linear, reproducible, robust, rugged and stability-indicating for simultaneous calculation. It can be used as a routine quality control method for combined pharmaceutical dosage form and for its kinetic studies.

Keywords: Development and validation of analytical method, Quantitative analysis of anticoagulants, Real-time valuation of drug-drug compatibility, Stability indicating HPLC method.

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Received: 18-12-2021;
Revised: 22-01-2023;
Accepted: 04-02-2023.

INTRODUCTION

Compliance of patient and drug-drug interaction are two considerable issues of present time especially in case of acute diseases. They also play a significant part in the increasing re-hospitalization of patients due to which cost of treatment is rising. Therefore, by keeping these issues into consideration the aim to prepare a combination of formulations with reduced possibility of drugs interaction and improved ability of compliance has been paramount among pharmaceutical researchers.

Aspirin (A) and Clopidogrel (CB) both are used as antiplatelet agent for the prevention of blood clotting. Clopidogrel is categorized as a BCS class II agent, which means it has poor water solubility and high permeability. As Per Literature survey regarding the treatment of cardiovascular disease,[1-5] Clopidogrel bisulfate shows better outcomes in patients as compared to aspirin and provides additional advantage to patients with acute coronary syndromes already taking aspirin.[6]

Aspirin is a Nonsteroidal anti-inflammatory drug, although classified as NSAID but may possess different chemical structure as compared to other drug of the same class. Along with treatment of pain, fever, and inflammation, it is also used to prevent clumping or clotting of blood cells as it fits into a BCS II drug class as well. It is supposed to be the safest and most effective medicine and present in World Health Organization's Essential Medicines.[7] On the other hand, higher dose of aspirin can cause chronic kidney disease, can also be a major reason of stomach pain, ulceration and other side effects. But in low dose effectively prevents cardiovascular events in patients with and without hypertension

The use of combination of 2 antiplatelet agents is more effective[8] as compared to single one. In combination therapy clopidogrel is as effective as compared with aspirin in minimizing the risk associated with cardiovascular events.[9]

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DOI: 10.5530/ijpi.13.2.037

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To get the accurate and precise dose, oral drug delivery systems in solid form is one of the best routes of choice. It has potential to provide therapeutic drug concentrations in the quantity at the specific site.[10] In this regard, combination therapy (two in one) in immediate release dosage form helps to minimize the frequency of drug administration as well as facilitate the maintenance of pharmacokinetic parameters within the satisfactory level of patient safety.

HPLC analytical method is frequently used for the separation and quantitative analysis of an analytes. Reproducibility and robustness of the results is providing confidence regarding the precision in quantitation. In pharmaceutical industry, stability indicating HPLC methods are not only used to determine potency and efficacy of the products but also use to estimate the quantitatively impurities of drug substances and drug products within and after the shelf-life of the drug during the storage.[11] The estimation of content and impurity are the major and critical quality attributes of the pharmaceutical dosage forms, as it is helpful in adopting the suitable and adequate closer and container system for the protection and handling of the drugs and its formulated products.

The main objective of the present study was to focus on the development and validation of HPLC analytical method for simultaneous quantitative estimation of aspirin and clopidogrel. The stability of these two drugs together is one of the major challenges, therefore in the purposed method, combination was tested for the degradation of drugs together under various stress conditions as per ICH guidelines.[12]

MATERIALS AND METHODS

Materials

Clopidogrel bisulphate (CB) was obtained as gift sample from Aurobindo Pharma, Hyderabad, India. Aspirin (A) was also acquired as gift sample from Faculty of Pharmacy (University of Karachi, Pakistan), the source of material was JQC (Huayin) Pharmaceutical Co., Ltd., China, octane-1-sulfonic acid sodium salt (Fisher Scientific), orthophosphoric acid (Merck), acetonitrile (Merck), double distill water HPLC grade (Merck) and distillation apparatus was used to prepare fresh water.

Instruments

The proposed study was achieved by using HPLC system that consisted of a LC-20AD liquid chromatography furnished with SPD-20A UV-vis detector. Chromatographic separations were done on C18 (4.6 x 150 mm, 5 µm) column which was connected with loop 20 µL and attached with HPLC-Dell system. Hot air oven (Pol-Eko, Poland), Electronic balance (Mettler Toledo, England), pH meter (Hanna).

Methods

Preparation of Mobile phase

Prepared a mixture of acetonitrile and buffer (Octane-1-sulfonic acid sodium salt) in the ratio of 35:65 v/v. The mobile phase was degassed after the filtration through millipore filter (0.45 µm pore size)

Preparation of Diluent

50% of acetonitrile was used as diluent.

Preparation of Standard stock solutions

Accurately weighed amount of Aspirin (75mg, Ref) and Clopidogrel (100 mg, Ref) powder were separately transferred in to 100 ml volumetric flask. Diluents were added and shaken for 5 min. After shaking, 4 ml of each standard solution was diluted to 50 ml to get the final working solution of 0.06mg/ml of aspirin (60 µg/ml) and 0.08mg/ml of clopidogrel (80 µg/ml) then filtered through 0.45µm filter paper.

Construction of Calibration curve

The calibration curve was plotted by using aspirin and clopidogrel solution separately and in combination. Two different series of solutions such as 60-0.117 µg/ml for aspirin and 80- 0.156 µg/ml for CB were made and absorbance of solutions was measured by HPLC at λ = 220 nm.

Development and Validation of HPLC method

The study was performed to develop a HPLC quantitative method to calculate A and CB potency in raw material and also in their product formulations. Initially, the selection, composition, and adjustment of mobile phase and determination of lambda (λ) were done by screening. After getting the preliminary data, the experimental work on HPLC was carried out isocratically at a flow rate of 1.3 mL/min. The run time obtained for A and CB are 4-5 min and 11-12 min. respectively after optimizing the process. Then the method was validated as per International Conference on Harmonization (ICH-1996).[12] The suitability of the method was verified by estimating different parameters, such as system suitability, specificity, range and linearity, limit of detection, limit of quantification, accuracy, precision, ruggedness and robustness.

Drug-drug Compatibility study

In advanced pharmacotherapy, especially in case of cardiovascular atherothrombotic diseases, oral antiplatelet drugs work as cornerstone. In acute coronary syndromes, especially for those who received coronary artery stents, aspirin and clopidogrel are essential components of medical therapy as well as in the secondary prevention of ischemic stroke.[13]

Compatibility study of aspirin with clopidogrel was carried out at elevated temperature (50±2°C) for 3 weeks.

Forced degradation studies

Forced degradation studies were conducted to determine the degradation pathways of drug substances and identify the impurities related to drug. These studies are effective for the validation of stability indicating method for monitoring possible changes to a drug product over the time.

Forced degradation studies of both the drugs (A and CB) were carried out under conditions of acid or base hydrolysis, oxidation, thermal and photolysis. Stock solution of Aspirin and Clopidogrel were prepared in the concentration of 1mg/ml each and were used for forced degradation studies.

RESULTS

The major emphasis of present study was to develop and validate a method for simultaneous estimation of aspirin and clopidogrel. The development and optimization of chromatographic conditions were done by the sequence of steps by using separate columns, mobile phases with different compositions, flow rates, wavelengths with different diluents. The data of initial studies were used to choose the optimize design for HPLC to get the acceptable separation of aspirin and clopidogrel from each other. The validation and system suitability of the optimized design was carried out by making standard solution of aspirin (0.06mg/ml) and clopidogrel bisulphate (0.08 mg/ml) separately as well as in combined solution (60/80 µg/ml).

Optimization of HPLC method of analysis

For the standardization of proposed HPLC method, number of parameters were tested to accomplish better and consistent efficiency of the chromatographic system. An isocratic elution was used to separate the drugs components. The basic component of mobile phase was octane-1-sulfonic acid sodium salt, the good point of this salt is pH = 5.5 to 7.5 (100g/L, H_2O, 20°C), water soluble and good for ion pair chromatography. The selection of this salt was done by considering the system suitability parameters like resolution factor (R_f) between peaks, tailing factor (T), number of theoretical plates (N), runtime and the cost effectiveness.

System suitability

The method was checked for its suitability by injecting six replicate injections of standard working solution of aspirin and clopidogrel separately as well as in combined solution form (60/80 µg/ml) (Table 1).

Linearity

For the acceptability of method assessment, the linearity between known concentration and their respective peak area were constructed. It was done over the range of 60-0.117µg/ml for aspirin and 80- 0.156 µg/ml for clopidogrel (Figure 1). Absorbance of the solutions were measured at λ = 220 nm. The suitability of analysis was also checked for its LOD and LOQ (Figure 2).

Accuracy

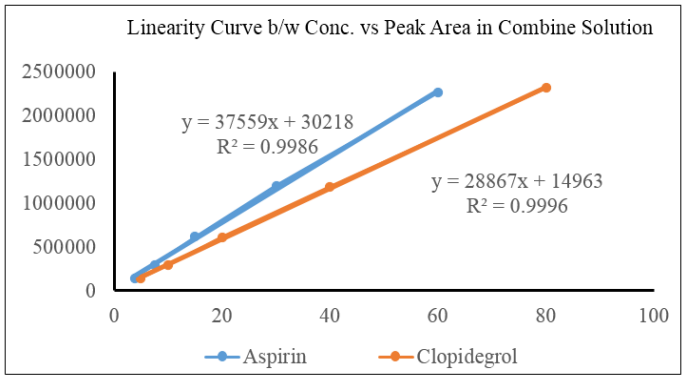
The accuracy of the method was estimated by recovery of aspirin and clopidogrel from their solution of three different concentrations over the range of 70-130% and analyzed as per method (Table 2).

Repeatability

(Precision) of the method was executed by injecting six (6) repeated samples of A and CB (Table 1) and three different concentrations, 60/80, 30/40 and 15/20 µg/ml at different time intervals (Table 3).

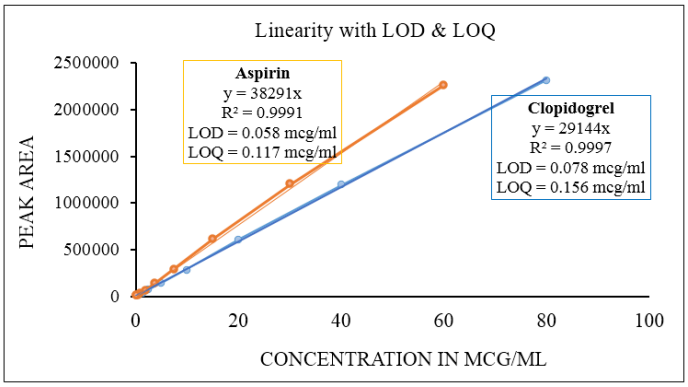
For drug-drug compatibility study

10 vials with 100mg of aspirin and 100 mg of clopidogrel bisulphate were prepared and kept in hot air oven. Analysis of these powder mixture was done periodically at 0, 1, 2 and 3 weeks (Table 4).



R_2 = Coefficient of determination

Figure 1: Calibration curve shows linearity over the concentration range of 60-0.117µg/ml for aspirin and 80- 0.156 µg/ml for clopidogrel.



*LOD = Limit of detection; LOQ = Limit of quantification

Figure 2: Estimation of LOD and LOQ of Aspirin and Clopidogrel.

Table 1: Summary of System suitability analysis for Aspirin and Clopidogrel.

Injection Number	Retention Time in minutes		Peak Area		USP Tailing Factor		USP Tangent		Resolution
	Aspirin	Clopidogrel	Aspirin	Clopidogrel	Aspirin	Clopidogrel	Aspirin	Clopidogrel	
1	4.306	12.592	2312961	2369195	1.246	1.381	8307.01	11678.99	6.325
2	4.32	12.622	2325419	2378773	1.249	1.381	8426.509	11842.174	6.367
3	4.332	12.738	2342735	2401344	1.259	1.385	8280.08	11937.57	6.407
4	4.391	12.918	2322485	2367106	1.241	1.382	8324.862	12090.343	6.479
5	4.221	12.676	2321247	2307940	1.24	1.377	8292.547	11973.671	6.254
6	4.222	12.69	2332745	2359453	1.234	1.361	8361.27	11793.49	6.289
Mean	4.299	12.706	2326265.333	2363968.5	1.245	1.378	8332.046	11886.0397	6.3535
± SD	0.067	0.116	10299.827	31025.293	0.0087	0.0087	54.252	145.213	0.082058
% CV	1.54	0.913	0.443	1.312	0.699	0.631	0.651	1.22	1.292

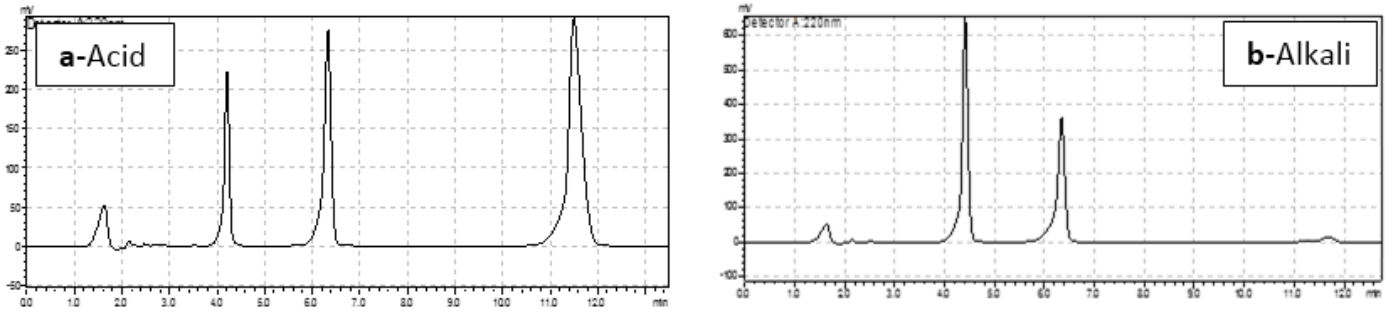


Figure 3: Chromatogram of a) Acid; b) Base degradation study.

Robustness study: It was conducted by making slight changes in the flow rate, concentration of acetonitrile and change in pH of mobile phase for the standard sample solution of concentration 60/80µg/ml (Table 5).

Forced degradation studies

In order to ensure that the method provides stability-indicating properties, forced degradation studies were performed under various stress conditions.

Acidic Hydrolysis

In 1.0 ml of stock solution (1mg/ml) added 10 ml methanol, 10 ml of 0.1N HCl then refluxed the mixture at 60°C for 6 hr. After neutralization to pH = 7 by 0.1 N NaOH, the solutions were injected into the HPLC to study the possibility of changes.

Alkaline Hydrolysis

Alkaline hydrolysis was performed by adding 10 ml methanol and 10 ml of 0.1N NaOH in 1.0 ml of stock solution (1mg/ml) then refluxed the mixture at 60°C for 6 hr. After neutralization to pH = 7 by 0.1 N HCl, the solutions were injected into the HPLC.

Oxidation degradation

oxidation decomposition study was performed in 3% v/v hydrogen peroxide (H_2O_2) solution at room temperature for 7 days.

Thermal degradation

It was performed by exposing drug substance (A and CB) at 100°C in hot air oven for 48 hr.

Photolytic degradation

Photolytic degradation study was carried out by exposing the drug substance (in solid form) to bright, sunny days from 11am to 5 pm for 4 days (24 hr).

DISCUSSION

Due to progress and advancement in the formulation of drug delivery system, the combination therapy products are frequently used to reduce the number of dose administration and to improve the patient compliance.[14] As per current guideline, it is recommended to use dual antiplatelet therapy (aspirin

Table 2: Accuracy data for the assessment of Aspirin and Clopidogrel.

Level	Sample Number	Theoretical Value Mg		Measured value mg		% Recovery		% Mean Recovery		% CV	
		Aspirin	Clopidogrel	Aspirin	Clopidogrel	Aspirin	Clopidogrel	Aspirin	Clopidogrel	Aspirin	Clopidogrel
70%	1	26.3	35	25.9	35.4	98.48	101.14	99.753	100.573	1.35	0.75
	2	26.1	34.8	26	35.1	99.62	100.86				
	3	25.9	35.3	26.2	35.2	101.16	99.72				
100%	1	37.7	49.8	37.6	50.1	99.73	99.6	100.99	100.863	1.21	1.12
	2	37.5	50.3	37.9	51.2	101.07	101.79				
	3	37.1	50.1	37.9	50.7	102.16	101.2				
130%	1	48.8	65.1	48.9	65.8	100.21	101.08	100.87	100.82	0.81	0.32
	2	47.9	65	48.75	65.6	101.78	100.92				
	3	48.7	65.2	49	65.5	100.62	100.46				
Mean								100.54	100.75	1.1233	0.73

Table 3: Analysis of Aspirin and Clopidogrel Bisulphate at different time interval (Intra-day study).

Conc. (µg/ml)	9.00 am		12.0 am		3.00 am		Mean		±SD		% CV	
	A	CB	A	CB	A	CB	A	CB	A	CB	A	CB
60/80	2301161	2359453	2358910	2398113	2301161	2321247	2339660	2359604	33341.4	38433.22	1.43	1.63
	2332745	2321247	2216718	2307940	2332745	2359453	2316903	2322880	4254.029	4472.953	0.184	0.193
Mean	2316953	2340350	2287814	2353026.5	2311204	2340350	2328282	2341242	18505.98	21352.23	0.795	0.91
30/40	1206357	1197600	1176162	1176360	1200521	1200495	1194347	1191485	16016.44	13178.37	1.34	1.11
	1194571	1187344	1200521	1200495	1203066	1202061	1198529	1196633	3427.753	8082.814	0.29	0.676
Mean	1200464	1192472	1188341.5	1188427.5	1200508	1201278	1196438	1194059	7011.665	6570.629	0.59	0.55
15/20	615353	607045	600518	601189	603980	602436	606617	603556.7	7761.097	3084.656	1.28	0.511
	618706	609808	613401	614124	604048	609682	612051.7	611204.7	7421.574	2529.002	1.21	0.414
Mean	617029.5	608426.5	606959.5	607656.5	604014	606059	609334.3	607380.7	6825.004	1207.612	1.12	0.199

±SD = Standard deviation (Measures the amount of variability)A= Aspirin; CB = Clopidogrel Bisulphate

and clopidogrel) for prevention and control of thrombotic complications especially after drug-eluting stents (DES) implantation for at least 1 year.[15]

In this scenario, it is important to ensure that the potency, efficacy and safety of APIs are as per specification in the dosage forms. The reliability of any research, developments and their establishment depend on a validated and accurate method of analysis to get a satisfactory and accurate result.

Optimization of HPLC method of analysis

Currently HPLC methods are predominantly used to estimate drug in bulk material or in finish products due to their fast and accurate approach towards method development and validation. In the present study, the details of the method development and validation along with key information regarding the degradation of aspirin and clopidogrel under different stress conditions are discussed.

System suitability

This test is done to check the performance of column. It is a fundamental part of method development. Theoretical plates, tailing factor, and retention time of samples verify the functioning of chromatographic system. The coefficient variation (%CV) of retention times and peak areas was 1.54 and 0.443% for aspirin and 0.913 and 1.312% for CB, respectively. The Mean theoretical plates count for A was 8332.046 and for CB was 11886.0397. Theoretical plates more than 2000 (A = 8332.046; CB = 11886.0397) describe the productivity of column, whereas the tailing factor less than 2.0 (A = 1.245; CB = 1.378), indicate that the peak is symmetrical (Table 1).

Linearity

Adjustment of procedure was started with the construction of calibration curve with linear relationship of two variable i.e, concentration vs peak area. It was done over the range of 60-0.117µg/ml for aspirin and 80- 0.156 µg/ml for clopidogrel. The regression analysis (R^2) was 0.9986 and 0.9996 for aspirin and clopidogrel respectively. The R^2 values indicate that the designated

Table 4: Drug-drug Compatibility study of Aspirin and Clopidogrel Bisulphate.

Concentration (µg/ml)	1st week		2nd week		3rd week	
	A	CB	A	CB	A	CB
60/80	2301161	2289453	2318310	2287653	2308910	2279859
	2332745	2311247	2376118	2318721	2320247	2308652
Mean	2316953	2300350	2347214	2303187	2314578.5	2294256
% CV	0.964	0.67	1.74	0.95	0.346	0.89
30/40	1206357	1201600	1169862	1200765	1210521	1199495
	1194571	1197344	1201421	1201128	1209875	1208761
Mean	1200464	1199472	1185641.5	1200946.5	1210198	1204128
% CV	0.694	0.25	1.88	0.021	0.038	0.544
15/20	615353	614045	610238	608231	610880	613624
	618706	609998	610134	618764	613458	609689
Mean	617029.5	612021.5	610186	613497.5	612169	611656.5
% CV	0.384	0.468	0.012	1.214	0.298	0.455

Table 5: Outcomes of Robustness study for A and CB.

Parameter	Changes	% Target	% Recovery	%CV
Target Conditions		**100.73**	**99.93**	**0.57**
Flow rate	1.0 ml/min	100.25	99.38	0.26
	1.3 ml/min	100.09	100.11	0.078
Buffer pH	2.9	101.5	100.4	0.73
	3.1	100.07	98.96	1.04
Acetonitrile Variation	32%	102.11	99.65	1.634
	38%	99.9	100.8	0.171

% CV = Coefficient of variation (help to precise the estimation)

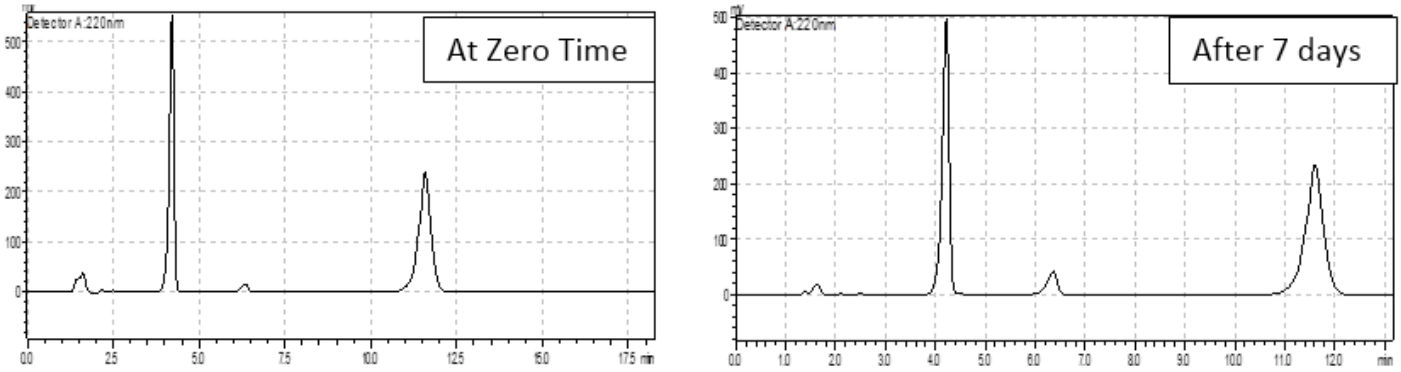


Figure 4: Chromatogram of Oxidation degradation study.

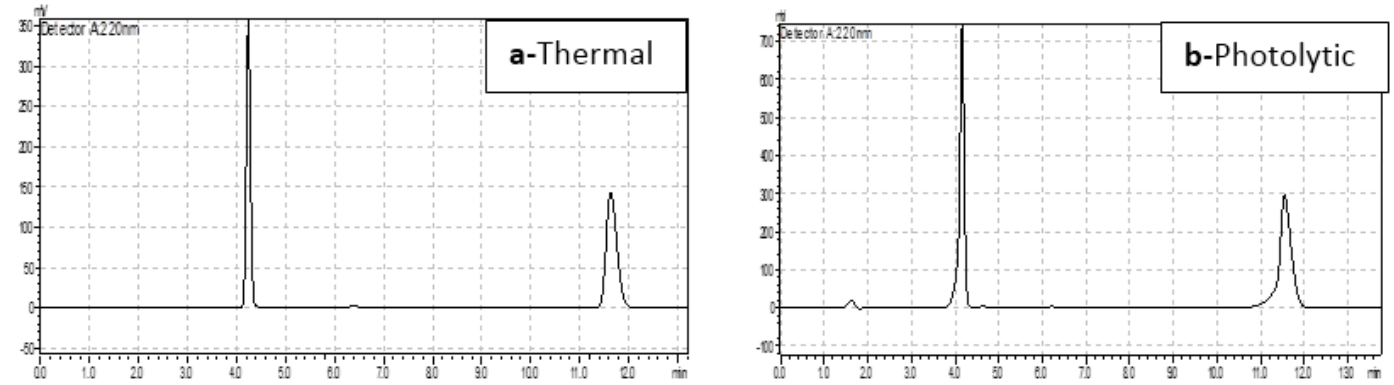


Figure 5: Chromatogram of, a) Thermal; b) Photolytic degradation study.

analytical procedure has capacity to support quantitative data with acceptable level of accuracy and precision (Figure 1).

To calculate the Limit of detection (LOD) and Limit of quantification (LOQ), the standard solution of both drugs together in the range of 60-0.0.058 µg/ml for aspirin and 80- 0.078 µg/ml for clopidogrel were made by serial dilutions and analyzed. Based on the signal: noise ratio, the LOD value for aspirin and clopidogrel were found as 0.058 µg/ml and 0.078µg/ml and the LOQ value were 0.117 µg/ml and 0.156 µg/ml for A and CB respectively that demonstrated the specified level of accuracy and precision of the method (Figure 2). These values of LOD and LOQ are supported with previously reported methods.[16-24]

Accuracy

The accuracy of the method was assessed by recovery of measured value from their solution. The mean recovery range for aspirin was 99.753 – 100.99% and 100.573 -100.82% for clopidogrel with % CV value of 0.81-1.35% and 0.32-1.12% respectively for A and CB (Table 2). The differences between theoretical and recovered values are not very significant. Therefore, the results demonstrate that the proposed method is fast and accurate for the simultaneous quantification of A and CB.

Precision

The method was exhibited for precision level at two separate stages, 1st was repeatability and 2nd were intermediate precision.[25] The 1st stage of precision helped to comprehend the working requirements of equipment over a short period of time. It is also termed as inter and intra-day assay.

The inter and intra-day variations of the method was checked by injecting combine solution (A + CB) of three levels of concentrations at different time intervals, within a day or in different days. The % CV was found less than 2% for both the drug substances which indicates high precision of method. The results of analysis also described that the samples are stable in their solution form even after three days (Table 3).

Intermediate precision

The method was carried out by different analyst in different laboratory by using different HPLC column and system (Faculty of Pharmacy, University of Karachi). The results of samples were calculated for % CV and were found between 0.28-1.76%. It indicated that the method effectively met the criteria of acceptance for inter-batch precision which is appropriately less than 15%.

Clopidogrel is extensively used in combination with aspirin to control the events that usually occur due to thromboembolism

in the arteries and veins. As literature shows that the aspirin has potential to hydrolyze and decompose very quickly, in these circumstances, it is important to analyze the level of decline of aspirin in the presence of clopidogrel and their possible interaction. In this regard, drug-drug compatibility study was conducted to assess the possibility of their decomposition in their combined state (Table 4). The study was done on three concentrations (60/80; 30/40 and 15/20) and their % CV was between 0.012 to 1.74 which described the good compatibility of these two compounds.

Robustness study

For more confirmation and verification of the method, robustness study was conducted on a sample concentration of 60/80µg/ml. The findings showed that the changes in the flow rate from 1.0 ml/min. to 1.3 ml/min, with % CV 0.26- 0.078, didn't reflect the major variations. Due to change in pH from 2.9 to 3.1, with coefficient variation of 0.73-1.04% and the variation in the concentration of acetonitrile from 32 to 38 indicated a recovery of 99.65 and 100.8% robustness with % CV of 1.634 and 0.171 respectively (Table 5).

Forced degradation studies

Aspirin is stable at a pH of 2-3. In alkalis solutions, aspirin decomposes rapidly due to hydrolysis and form clear solution of acetate and salicylate.[26] The combination of aspirin and clopidogrel is used in dual antiplatelet therapy. In order to investigate the stability level of combine therapy, it is essential to have an analytical method that should have potential to estimate the trace amount of any impurities that can be produced during the shelf-life of the drug substance. Beside this, ICH Regulatory guidance[12] (Q2A, Q2B, Q3B and FDA 21 CFR section 211) also notify the need of development and validation of stability indicating analytical method.

For the estimation of acid decomposition, stock solution (1mg/ml) of aspirin and clopidogrel was exposed to acidic environment and quantitative assessment was done by using intended HPLC analytical method (Figure 3a). It was observed that aspirin (A) was more susceptible to acid hydrolysis. Degradation was observed by the decreased in peak area (22.05%) of the drug substance with one additional peak at 6.3 min. This finding is supported by previous reported study.[27,28] Whereas on the other hand, both the drugs (A and CB) were sensitive to alkali hydrolysis which was indicated with one additional peak at 6.3 min with 36.963% decomposition of aspirin peak area and only 4.65% clopidogrel was left after one hour of stress condition (Figure 3b).

In case of Oxidation degradation, no significant changes were observed in the peak area of both the drugs after 7 days (Figure 4). Only slight change was observed in the peak area of aspirin (12.47%). This result is endorsed by the study.[27]

Thermal degradation

was performed at 100°C in hot air oven. The resultant chromatogram indicated that both aspirin and clopidogrel are stable under thermal condition (0.0% decomposition) (Figure 5a). Although the photolytic degradation study was done by exposing the substance (in solid form) in sunlight (6hr/day) for 4 days (Figure 5b). In this way, the drug molecules underwent the photolytic degradation depending on the intensity of sunlight. It was observed that the drug molecules were more stable under photolytic degradation as compared to other stress conditions. It was also observed that aspirin is more stable under sunlight as compared to clopidogrel (slight peak fronting) as shown in Figure 5b. This finding is supported by previous reported study.[27]

CONCLUSION

The present study was conducted to establish a new HPLC analytical method for the simulation estimation of aspirin and clopidogrel. The method is simple, precise, accurate, rapid and stability-indicating that can facilitate the estimation of both drugs (A and CB) in short period of time. The stability-indicating nature of the proposed method was established by performing forced degradation, which provided the idea regarding the degradation behavior of Aspirin and Clopidogrel under various conditions. The developed method was validated as per ICH guidelines. The method can be used for routine analysis of clopidogrel and aspirin.

ACKNOWLEDGEMENT

The authors would like to thank Dr. S. Gurumadhva Rao (President), Dr. Padma Rao (Dean) and Dr. Venkat Ramesh (Associate Dean), RAK College of Pharmacy, RAK Medical and Health Sciences University, Ras Al Khaimah, for continuous encouragement and support.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ICH: International Conference on Harmonization; **HPLC:** High-performance liquid chromatography; **A:** Aspirin; **CB:** Clopidogrel; **BCS:** Biopharmaceutics Classification System; **LOD:** Limit of Detection; **LOQ:** Limit of Quantitation; **APIs:** Active Pharmaceutical Ingredient; **CV:** Coefficient Variation; **R^2**: Regression Analysis.

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Cite this article: Usman S, Akram M, Shah F, Ramesh KVRNS, Islam Q. Stability Indicating HPLC Method for Simultaneous Assessment of Clopidogrel Bisulfate and Aspirin: Development and Validation. Int. J. Pharm. Investigation. 2023;13(2):270-8.